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Disinfection By-Products: Formation In Pool Water And The Role Of Iodinated Medical Imaging Compounds As A Potential Precursor In The Formation Of IODO-DBPS

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DISINFECTION BY-PRODUCTS: FORMATION IN POOL WATER AND THE ROLE OF IODINATED MEDICAL IMAGING COMPOUNDS AS A POTENTIAL PRECURSOR IN THE FORMATION OF IODO-DBPS

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DEDICATION

In dedication to my parents, my sister, and Rascal for being my biggest fans always. I would not be where I am without your love, support, and endless encouragement.

ACKNOWLEDGEMENTS

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ABSTRACT

Disinfection by-products (DBPs) are the unintended consequence of using chemical disinfectants to kill pathogens in water. [1-11] They are formed from the reaction between disinfectants and natural organic matter (NOM), anthropogenic contaminants, and bromide/iodide present in the raw source water. DBPs are cause for concern because studies have found them to be toxic.[11-16] This research focuses on identifying and studying the formation of DBPs. Specifically, the presence of DBPs in chlorinated pool water and tap water from Barcelona, Spain was studied to better understand which DBPs are formed in the pool as compared to those that enter the pool from the tap water source used to fill the pool. Samples from the pool and tap were analyzed using gas chromatography-mass spectrometry (GC-MS) and approximately 100 DBPs were comprehensively identified. It was found that more nitrogen-containing DBPs (N-DBPs) were identified in the pool water than in the tap water, likely due to the increased nitrogenous input from swimmers in the pool (urine, sweat, skin cells, hair,cosmetics). Additionally, this research evaluated the role of X-ray contrast media (ICM) as a precursor for iodo-DBPs, by studying source waters from several locations that had been reacted with ICM and chlorine disinfectant.

Several iodo-DBPs were found in the iopamidol-chlorinated waters, including five new iodo-DBPs not previously known: iodoacetonitrile, chloroiodoacetonitrile, dichloroiodoacetic acid, bromochloroiodoacetic acid, and chlorodiiodoacetic acid. Gas chromatography (GC)- high resolution mass spectrometry (HRMS) (with electron ionization [EI]) was used to identify the new iodo-nitriles, and a new, sensitive GC-EI-MS/MS method was used to identify the new iodo-acids.

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LIST OF ABBREVIATIONS

AOX..... Adsorbable Organic Halogens

BCIM ..... Bromochloroiodomethane

BDIM..... Bromodiiodomethane

CDIM ..... Chlorodiiodomethane

DBIM..... Dibromoiodomethane

DBPs..... Disinfection By-Products

DCIM ..... Dichloroiodomethane

DM..... Diazomethane Derivatized Extract

EA ..... Ethyl Acetate Extract

EI..... Electron Ionization

GC..... Gas Chromatography

GC-MS ..... Gas Chromatography-Mass Spectrometry

HAAs ..... Haloacetic Acids

HRMS..... High Resolution Mass Spectrometry

HR-TOF-GC-MS..... High Resolution Time of Flight GC-MS

IAN..... Iodoacetonitrile

ICM..... X-ray Contrast Media

LC-MS/MS..... Liquid Chromatography-Mass Spectrometry/Mass Spectrometry

LLE ..... Liquid-Liquid Extraction

MRM .....Multiple Reaction Monitoring

MX .....Mutagen X

N-DBPs ..... Nitrogen-containing Disinfection By-Products

NIST ..... National Institute of Standards and Technology

NOM .....Natural Organic Matter

ppb ..... parts per billion

SIM .....Selected Ion Monitoring

THMs .....Trihalomethanes

TIM ..... Triiodomethane

TOX ..... Total Organic Halogens

USEPA .....United States Environmental Protection Agency

CHAPTER 1

INTRODUCTION

Since the 1900s, water disinfection has played an important role in decreasing the prevalence of waterborne illnesses that were once a leading cause of death among the population.[17] Disinfectants used vary by location, but include chlorine, chloramine, chlorine dioxide, and UV Treatment.[11] While the disinfectants inactivate pathogens such as salmonella typhi, vibrio cholerae, shigella, E.coli, etc. that are responsible for diseases such as typhoid fever, cholera, and dysentery, the disinfection process is not without its drawbacks.[11]

The strong oxidizing capacity of the disinfectants cause the death of microorganisms that are responsible for many diseases. [5, 12, 18-19] However, the strong oxidizing power is not selective, and the disinfectants will also react with natural organic matter (NOM), anthropogenic contaminants, and bromide/iodide present in the raw source water to form disinfection by-products (DBPs). [11-16] Thus, disinfection by-products are the unintended consequence of using chemical disinfectants to kill pathogens in water, and have been reported to form with most disinfectants.[1-11]

DBPs are a source of concern because studies have linked them to bladder cancer, early term miscarriages, birth defects, asthma, and other respiratory ailments. [2, 4-5] Trihalomethanes (THMs) and haloacetic acids (HAAs) are two of the most prevalent groups of DBPs formed during disinfection.[4-5, 7, 20-21] Because of their implications on human health, the US Environmental Protection Agency (USEPA) has set regulatory limits on four THMs and five HAAs.[5, 10, 22] However, disinfection can also form many more DBPs than those that are regulated. In the presence of bromide and iodide in the water, chlorine can react to form not just chlorinated DBPs, but also brominated and iodinated-DBPs.[1, 13, 23-25] Iodinated DBPs have been found to be more toxic than their brominated and chlorinated analogues and nitrogen containing DBPs (N-DBPs) have been found to be more toxic than their carbon-based analogues (Figures 1.1 and 1.2) [23, 26-27]

There are many factors that contribute to the formation of different DBPs, including concentrations of bromide/iodide in the source water, concentration and composition of natural organic matter, and temperature.[6, 10, 22] The presence of DBPs in drinking water is well studied, but it is hypothesized that only a small percentage have been identified.[2, 10-11, 22, 28] Thus, in order to get a better understanding on the formation of DBPs and further identify the unknowns, it is important to study environments that have characteristics that will likely

promote the formation of DBPs as well as investigate potential precursors of DBPs.

Specifically, this research investigates the occurrence of brominated and chlorinated DBPs found in a chlorinated swimming pool (Chapter 2) and the role of iodinated X-ray contrast media (ICM) as a precursor to iodinated DBPs found in drinking water (Chapter 3)

FIGURES

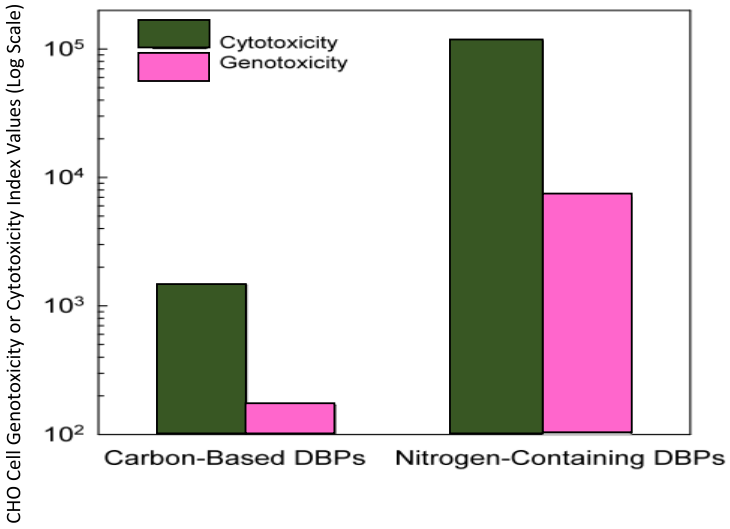

Figure 1.1: Cytotoxicity and genotoxicity of carbon-based DBPS compared to nitrogen-based DBPs.[29]

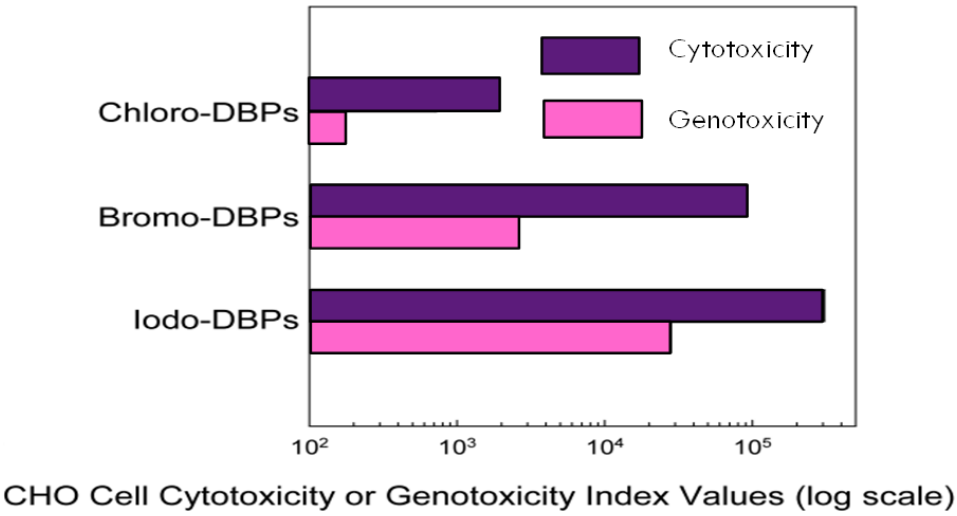

Figure 1.2: Cytotoxicity and genotoxicity of halogenated DBPs.[29]

CHAPTER 2

COMPREHENSIVE IDENTIFICATION OF DISINFECTION BY-PRODUCTS IN POOL AND TAP WATERS FROM BARCELONA, SPAIN

2.1 Introduction

The same factors that contribute to DBP formation in drinking water also exist in pools. Such factors include the presence of a disinfectant, exposure to high temperatures, and organic input from swimmers (urine, sweat, cosmetics, skin cells, hair).[4, 9, 16, 28, 30-43] This research has important implications because prior studies have shown that inhalation and dermal contact are significant routes of exposure to carcinogenic and irritant DBPs.[3-4, 6-7, 21, 28, 41, 44-48] Some studies even suggest that exposure from inhalation and dermal absorption may be equivalent to or greater than that from ingestion.[5, 44-46, 49] Biomarkers of lung epithelium permeability (serum CC16) and genotoxicity (micronuclei [MN] in lymphocytes) and urine mutagenicity were previously found to be related to brominated THM exposure subsequent to swimming 40 minutes in a chlorinated indoor pool. Another study showed that after swimming, the total concentration of four THMs in exhaled breath was seven times higher than before swimming.[50] Given

the high nitrogen content of organic matter from swimmers, nitrogenated species, such as haloacetonitriles and haloamides, are found in pools. As mentioned previously, nitrogen-containing DBPs are more toxic than their carbon-container analogs. Studies have suggested that asthma and other respiratory ailments in swimmers and pool workers have a likely association with DBPs found in the pool.[51-54] Thus, these toxic DBPs pose a threat to swimmers and therefore need to be identified.[50, 55-56] Chapter 1 discusses research focused on the identification of brominated and chlorinated DBPs in both tap and pool water from Barcelona, Spain.

2.2 Experimental Methods

Water Sampling. Collaborators in Barcelona, Spain conducted four sampling campaigns on four different days (June 3, 2013 [pool/tap 1], June 11, 2013 [pool/tap 2], November 18, 2013 [pool/tap 3], and December 9, 2013 [pool/tap 4]). During each sampling event, water was collected from the same pool and corresponding tap water in the pool facility. Two-liter Teflon bottles were used to collect 25 L of each (headspace free). Samples were stored at 4°C and shipped overnight in coolers with icepacks to the United States. Water samples were extracted immediately upon arrival using XAD resin extraction.[57] After

extraction, pool and tap water samples were sent to our lab for comprehensive gas chromatography-mass spectrometry analysis.

Comprehensive GC-MS Analysis. Analyses were performed using electron ionization (EI) on an Agilent 5975 inert XL Mass Selective Detector mass spectrometer equipped with an Agilent model 6890 GC and operated at an accelerating voltage of 8 kV and a source temperature of 200°C (Agilent Technologies, Santa Clara, CA). Injections of 1 μL of the extracts were introduced via a split/splitless injector (in pulsed splitless mode) onto a GC column (RTX-200, 30 m X 0.25 mm i.d., 0.25 μm film thickness, Restek, Bellefonte, PA). The GC temperature program consisted of an initial temperature of 40°C (4 min), increased to 9°C/min to 280°C and held for 20 minutes, for a total run time of 50.67 minutes. The transfer line was held at 280°C and the inlet temperature at 250°C with an electron energy at 70 eV. Analyses were performed in full scan. Example chromatograms for tap 4 ad pool 4 are illustrated in Figures 2.1 and 2.2

2.3 Results and Discussion

Approximately 100 DBPs were comprehensively identified in the tap and pool water (Table 2.1) from Barcelona, Spain. Such classes of DBPs included halomethanes, haloacids, haloacetonitriles, haloaldehydes, haloketones, halonitromethanes, haloamides, haloalcohols, and halophenols, where either

bromine, chlorine, or both were the halogen present. No iodinated DBPs were detected in the tap or swimming pool waters.

In general, the profile of identified DBPs was similar in both the tap and pool water samples. For example, all samples contained dibromochloromethane, tribromomethane, bromodichloroacetaldehyde, dibromoacetaldehyde, dichloroacetic acid, dibromoacetic acid, trichloroacetic acid, bromodichloroacetic acid, dibromochloroacetic acid, and benzaldehyde. However, there were a few compounds that were identified solely in the tap water samples or solely in the pool water samples. Several tribrominated compounds were found only in the tap water samples. Such compounds included tribromoacetonitrile, tribromoacetaldehyde, tribromoacetic acid, and 1,1,1-tribromopropanone found in tap 1, 3, and 4. Other compounds identified only in the tap water sample include 1-bromo-2-propanone, 2,2-dibromopropanoic acid, cis-2,3-dibromobutenedioic acid, and trans-2,3-dibromobutenedioic acid. Tri-brominated compounds are highly subject to degradation, so it is probable that the ones identified in the tap water degraded before entering the pool, and thus were not identified in the pool waters.

In the pool samples, bromodichloromethane was a uniquely identified DBP, as was 1-bromo-1-chloropropanone, and chloroacetic acid in pool 2, 3, and 4. In general, there were more N-DBPs found in the pool water samples than

their corresponding tap water samples. For example, dichloroacetonitrile was found in every pool water sample, but was not present in tap 1 or tap 4. Dichloronitromethane and bromodichloronitromethane were both present in every pool sample, but absent from every tap water sample. Similarly, trichloronitromethane was detected in every pool water sample, but only found in tap 2. Dichloroacetamide and trichloroacetamide were both present in every pool sample and not present in any tap water sample. Bromochloroacetamide was found in pool 2 and 3, and dibromoacetamide was found in pool 3, but neither were found in any tap water samples. The greater presence of N-DBPs in the pool samples as compared to the tap water samples was not surprising because pools have a greater contribution of nitrogen-containing precursors from human inputs, such as hair, urine, sweat, skin cells, and cosmetics.

DBPs identified in the swimming pool water formed from the reaction of the chlorine disinfectant with natural organic matter found in the pool. DBPs identified solely in the tap water samples formed from the initial reaction of disinfectant with natural organic matter in the drinking water plant. These particular chemicals potentially could have volatilized upon entering the pool and thereby were not also identified in the pool water. Additionally, Barcelona has a high amount of bromide in their source water, which causes more brominated DBPs in the tap water samples. By the time the tap water reaches the

pool facility, the bromide is likely all consumed. This, along with the chlorination of the pool water, and potential degradation, is a plausible explanation for why there are more brominated compounds in the tap samples that were not identified in the pool samples. DBPs identified in both the tap and the pool water would need further quantification analysis to see if the quantity present in the pool water was the same as in the tap water, or if more had formed in the chlorinated pool.

2.4 Conclusions

Around 100 DBPs were comprehensively identified in tap water and pool water samples collected from the same pool and tap during four sampling campaigns in Barcelona, Spain. While the DBPs identified in the tap water samples and pool water samples had many similarities, there was a prevalence of N-DBPs in the pool water samples, likely from the increased nitrogenous organic input from swimmers.

FIGURES

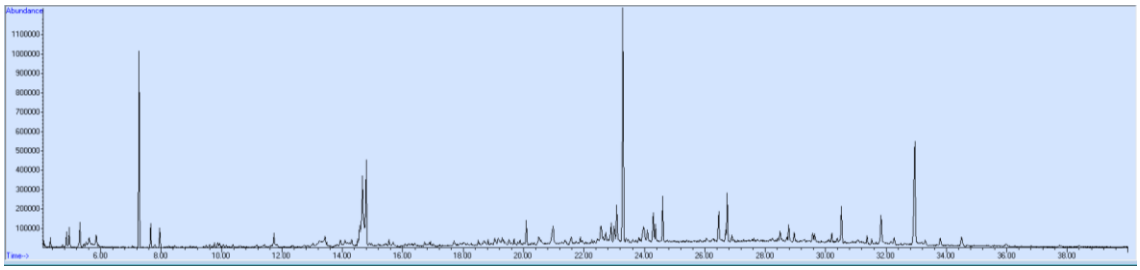

Figure 2.1: GC-MS chromatogram of tap 4, tap water sampled from Barcelona, Spain on December 9, 2013.

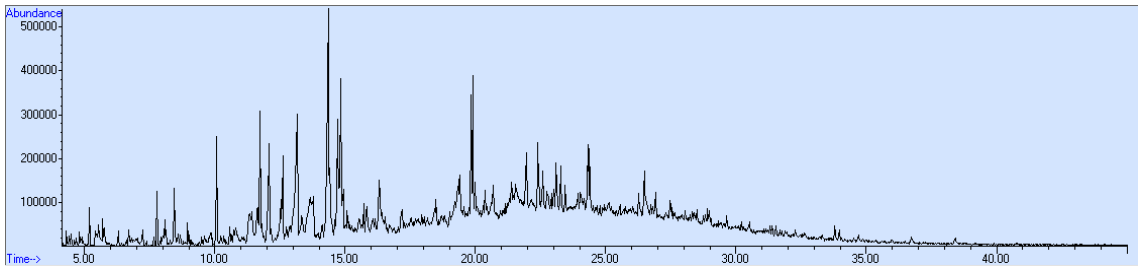

Figure 2.2: GC-MS chromatogram of pool 4, pool water sampled from chlorinated indoor pool in Barcelona, Spain on December 9, 2013.

TABLES

Table 2.1: DBPs identified in pool and tap waters. "EA" denotes extracts in ethyl acetate and not derivatized. "DM" denotes extracts that were derivatized with diazomethane.

	Tap 1 EA	Pool 1 EA	Tap 1 DM	Pool 1 DM	Tap 2EA	Pool 2 EA	Tap 2 DM	Pool 2 DM	Tap 3 EA	Pool 3 EA	Tap 3 DM	Pool 3 DM	Tap 4 EA	Pool 4 EA	Tap 4 DM	Pool 4 DM
Regulated Trihalomethanes																
Bromodichloromethane		X				X				X		X		X		X
Dibromochloromethane	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Bromoform	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Unregulated Haloalkanes																
Bromomethane											X		X			
Dibromomethane											X					
2,3-Dichlorooctane					X		X									
2-Bromo-1-chloro-2-methylpropane		X		X				X								
2-Bromo-1-chloro-2-propane						X										
1,1-Dichloropentane					X	X										
1,1,2-Trichloroethane	X								X		X		X		X	
Halonitriles																
Dichloroacetonitrile		X		X	X	X	X	X	X	X	X	X		X	X	X
Bromochloroacetonitrile	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Dibromoacetonitrile	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tribromoacetonitrile	X		X		X		X		X		X		X		X	

"X" indicates that a particular DBP was identified in that sample.

Table 2.1 continued

	Tap 1 EA	Pool 1 EA	Tap 1 DM	Pool 1 DM	Tap 2EA	Pool 2 EA	Tap 2 DM	Pool 2 DM	Tap 3 EA	Pool 3 EA	Tap 3 DM	Pool 3 DM	Tap 4 EA	Pool 4 EA	Tap 4 DM	Pool 4 DM
Haloketones																
1-Bromo-2-propanone	X		X		X		X		X		X		X		X	
1-Chloropropanone														X		X
1,1-Dichloropropanone					X					X		X				
1,3-Dichloropropanone												X				
1-Bromo-1-chloropropanone		X				X		X		X		X		X		
1,1-Dibromopropanone	X		X													
1,1,1-Trichloropropanone		X		X	X	X	X	X	X	X	X	X	X	X	X	X
1,1,3-Trichloropropanone						X				X		X		X		X
1-Bromo-1,1-dichloropropanone		X			X	X	X		X	X	X		X		X	
1,1,1-Tribromopropanone	X		X						X		X		X		X	
1,1,1,3-Tetrachloropropanone		X		X	X	X	X	X	X	X	X	X	X	X	X	X
1,1,3,3-Tetrachloropropanone		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Pentachloropropanone					X	X	X	X	X	X	X	X		X		X
3-Chloro-2,5-furandione		X		X	X	X	X	X		X		X		X		X
3,4-Dichloro-2,5-furandione								X		X		X				
1-Chloro-3,3,-dimethyl-2-butanone		X				X										
Haloaldehydes																
2,3-Dichloro-2-methylpropanal														X		
2-Bromo-2-methylpropanal								X								
Bromochloroacetaldehyde	X	X	X	X	X	X	X	X	X		X		X	X	X	
Bromodichloroacetaldehyde	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Dibromoacetaldehyde	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Dibromochloroacetaldehyde	X		X		X		X		X		X		X		X	
Dichloroacetaldehyde		X	X		X	X	X	X	X	X	X	X	X	X	X	X
Tribromoacetaldehyde	X		X		X		X		X		X		X		X	
Trichloroacetaldehyde		X		X	X	X	X	X		X		X		X		
Halonitromethanes																
Dichloronitromethane		X		X		X		X		X		X		X		X
Trichloronitromethane		X		X	X	X	X	X		X		X		X		X
Bromodichloronitromethane		X		X		X		X		X		X		X		X
Haloamides																
Dichloroacetamide		X		X		X		X		X		X		X		X
Bromochloroacetamide								X		X						
Dibromoacetamide										X		X				
Trichloroacetamide		X		X		X		X		X		X		X		X
Haloacetic Acids																
Chloroacetic acid								X				X				X
Dichloroacetic acid			X	X			X	X			X	X			X	X
Bromochloroacetic acid			X				X	X			X				X	X
Dibromoacetic acid			X	X			X	X			X	X			X	X
Trichloroacetic acid			X	X			X	X			X	X			X	X
Bromodichloroacetic acid			X	X			X	X			X	X			X	X
Dibromochloroacetic acid			X	X			X	X			X	X			X	X
Tribromoacetic acid			X				X				X				X	

Table 2.1 continued.

	Tap 1 EA	Pool 1 EA	Tap 1 DM	Pool 1 DM	Tap 2EA	Pool 2 EA	Tap 2 DM	Pool 2 DM	Tap 3 EA	Pool 3 EA	Tap 3 DM	Pool 3 DM	Tap 4 EA	Pool 4 EA	Tap 4 DM	Pool 4 DM
Other Mono-Haloacids																
2,2-Dibromopropanoic acid			X				X				X				X	
2,2-Dichloropropanoic acid				X			X	X			X	X			X	X
2,3-Dichloropropenoic acid												X				
2-Chloro-3-methylbutanoic acid				X				X								X
2-Chloro-3-methylmaleic acid			X		X		X				X					
Bromomaleic acid	X				X		X				X		X		X	
cis-2,3-Dibromobutenoic acid			X				X	X			X				X	
Dibromomaleic acid					X		X				X		X			
trans-2,3-Dibromobutenoic acid			X				X	X			X				X	
Trichloropropenoic acid				X				X			X	X			X	X
Halo-di-acids																
cis-2,3-Dibromobutenedioic acid			X				X				X				X	
trans-2,3-Dibromobutenedioic acid			X				X				X				X	
Other Halogenated DBPs																
1,1-Dichloro-1-propene						X										
1,2-Dichloro-1-propene						X										
1,2-Dichloroethanol acetate														X		
1-Bromo-2-methylbenzene		X											X		X	
1-Bromo-3,5-dimethylbenzene					X											
1-Chloro-2-ethanol acetate								X		X		X	X	X	X	X
2,2-bis (chloromethyl)-1-propanol										X						
2,3-Dichloropropanol					X											
2,4-Dibromo-2-butyne									X							
2-Bromo-1,1-diethoxyethane													X			
2-Chloro-1, 1-diethoxyethane					X	X		X	X				X			
2-Chloro-1,3-butadiene												X				
2-Chloroethyl benzoate										X		X				
3,3,3-Trichloropropene						X										
3-Chloro-1-hexene	X		X		X		X									
3-Chloro-2-methyl-1-propene												X		X		
6-Chloro-1-hexanol													X			
Benzyl chloride		X												X		
Benzyl chloroformate		X														
Bromomethyl benzene									X	X			X	X		
Dichlorophenol																X
Tetrachlorobenzenamine						X										
Tetrachlorophenol												X				
Tribromophenol													X		X	
Trichlorobenzenamine		X		X		X								X		X
Trichloromethylbenzene						X										
Trichlorophenol		X		X		X		X		X		X		X		X
Non-Halogenated DBPs/Chemicals																
Benzaldehyde	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

CHAPTER 3

IODINATED MEDICAL IMAGING COMPOUNDS AS A POTENTIAL PRECURSOR FOR IODINATED DISINFECTION BY-PRODUCTS IN DRINKING WATER

3.1 Introduction

When chlorine is used as the disinfectant, the reaction of free chlorine with NOM forms chloro-DBPs. Simultaneously, the free chlorine can react with iodide present to rapidly form hypoiodous acid. The hypoiodous acid can then react with NOM to form iodo-DBPs. This reaction, however, is not as kinetically favored as the reaction between hypoiodous acid and free chlorine, which reacts to form iodite, and then further reacts with more free chlorine to form iodate. The iodate is a non-toxic sink for iodide. Thus, iodo-DBPs are much less prevalent when chlorine is used as the disinfectant (Figure 3.1).[58] Conversely, when monochloramine is used as the disinfectant, the monochloramine will also react with NOM to form chloro-DBPs, but the reaction between the monochloramine and iodide to form hypoiodous acid is much faster. Unlike chlorine, monochloramine does not rapidly oxidize hypoiodous acid into iodite. While the reaction is thermodynamically possible, it is not kinetically favored.

This causes hypoiodous acid to have a longer half-life in waters that are disinfected with monochloramine, which gives them time to react with NOM and form iodo-DBPs (Figure 3.2).[58] Iodo-DBPs are therefore the toxic sink for iodide in this reaction. Although the formation of iodo-DBPs is favored with monochloramine disinfection, disinfection with monochloramine generally produces lower levels of the regulated trihalomethanes, haloacetic acids, and total organic halogen when compared to free chlorine.[1] Therefore, many cities are switching from chlorination disinfection to chloramination.[1]

A 23 city survey was conducted in the United States and Canada to investigate the occurrence of iodo-DBPs.[1] It was found that generally, in cities where there was high iodide naturally present, there were high concentrations of iodo-DBPs. There were four cities that did not follow this trend, however, and despite having low or non-detectable levels of naturally occurring iodide present, they still had higher (μg/L) concentrations of iodo-acids and iodo-THMs (Table 3.1). It was surmised that there must be another source of iodine that was contributing to the formation of these iodo-DBPs.[1]

X-ray contrast media (ICM) are widely used to enhance the visibility of soft tissues within the body for medical imaging.[59] They are non-toxic and after application, 95% of unmetabolized ICM are excreted from the body within 24 hours.[59] They are the primary contributor to the total organic halogen/adsorbable

organic halogens (TOX/AOX) burden in clinical wastewater and act as a large source of AOX in municipal wastewater.[60] In fact, ICM are found in the environment at higher concentrations than any other pharmaceutical.[60] In the four cities mentioned above with low naturally occurring iodide, there were high levels of ICM present in the waters (Table 3.2). [59] Iopamidol was present in the highest concentrations, suggesting that it could be a potential precursor for iodo-DBPs. It was found that in the presence of NOM, iopamidol could act as an organic iodine source and react with either chlorine or chloramine to produce iodo-DBPs.[61] Unlike iodide, however, iopamidol reacts more readily with chlorine, due to a different mechanism of formation.[59]

Using high resolution liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) high molecular weight iodo-DBP transformation products from iopamidol were isolated.[61-62] Genotoxicity and cytotoxicity testing were conducted on five of the 19 isolated high molecular weight DBPs, and it was found that they were not very toxic, especially in comparison to the lower molecular weight iodo-DBPs (Figure 3.3, Table 3.3).[62] However, increased toxicity of water when iopamidol is present was observed (Figures 3.4 and 3.5). It is hypothesized that the lower molecular weight DBPs being formed with iopamidol are driving the toxicity and are therefore crucial to identify. Chapter 2 focuses on research involving the role of iopamidol as a precursor to iodinated

DBPs, specifically with regard to the formation, identification, and quantification of lower molecular weight iodinated-DBPs in water from North Carolina (Cape Fear River), South Carolina (Broad River), and Germany (Rhine River).

3.2 Experimental Methods

Chemicals and Reagents. Reagents were ACS reagent grade and were purchased from Sigma-Aldrich (St. Louis, MO) and Fisher Scientific (Waltham, MA). DBP standards were purchased or custom synthesized from Sigma-Aldrich, CanSyn Chem. Corp. (Toronto, ON), Aldlab Chemicals (Woburn, MA), and TCI America (Waltham, MA) at the highest level of purity. Both 1,2-dibromopropane, internal standard, and Diazald, methylating agent for halo-acids, were purchased from Sigma-Aldrich. All solvents (acetonitrile, hexanes, methyl *tert*-butyl ether (MTBE), methanol, and ethyl acetate) were of highest purity and were purchased from Sigma-Aldrich (St. Louis, MO) or VWR International (Radnor, PA). Figures 3.6-3.8 give a summary of all experiments performed on each source water.

Comprehensive Identification Experiments. Water samples for comprehensive analysis were extracted using XAD resins (DAX-8 over XAD-2, 45 mL of each for North Carolina, 30 mL of each South Carolina, Germany), according to a previously published procedure.[57] Prior to use, new resins were

extensively cleaned using Soxhlet extraction.[63] Table 3.4 provides reaction and experimental conditions for each of the three different water sources. Briefly, water samples were acidified to pH 1 with sulfuric acid and were poured over the XAD resins packed in a glass column containing glass wool at the top and bottom. Once the water had completely eluted, organic compounds were eluted with ethyl acetate (420 mL for North Carolina, 100 mL for South Carolina, Germany). A separatory funnel was used to remove the water layer, and 30 g of sodium sulfate was used to further dry the extract. Final extracts were concentrated using a Turbovap and a gentle stream of nitrogen to1 mL and spiked with 1,2-dibromopropane internal standard. Half of the final XAD resin extract was analyzed directly by GC-MS and the other half was derivatized with diazomethane for the identification of halo-acids through their corresponding methyl esters.[55] Diazomethane was freshly generated according to a U.S. Environmental Protection Agency Standard Operating Procedure.[64] Briefly, approximately 0.367 g of Diazald® and 1.0 mL of CARBITOL™ were added to the inner tube of an Aldrich® diazomethane-generator apparatus and 3.0 mL of MTBE was added to the outer portion. The apparatus was assembled, placed in ice, and 1.5 mL of 37% potassium hydroxide (KOH) was injected dropwise through the septum into the inner tube. After reacting for 1 hour, 250 μL of the diazomethane (dissolved in MTBE in the outer tube) was added to each 500 μL

sample. After 30 min of reaction, excess diazomethane in the samples was quenched with approximately 10 mg of silica gel. Derivatized extracts were transferred to new vials to remove solid silica from the samples.

Comprehensive GC-MS analyses were performed using electron ionization (EI) on an Agilent 5975 inert XL Mass Selective Detector mass spectrometer equipped with an Agilent model 6890 GC and operated at an accelerating voltage of 8 kV and a source temperature of 200°C (Agilent Technologies, Santa Clara, CA). Injections of 1 μL of the extracts were introduced via a split/splitless injector (in pulsed splitless mode) onto a GC column (RTX-200, 30 m X 0.25 mm i.d., 0.25 μm film thickness, Restek, Bellefonte, PA). The GC temperature program consisted of an initial temperature of 35°C (4 min), increased to 9°C/min to 280°C and held for 30 minutes. The transfer line was held at 280°C and the inlet temperature at 250°C with an electron energy at 70 eV. Analyses were performed in full scan.

Samples from North Carolina and South Carolina were also analyzed on a LECO GC-HRT high resolution time-of-flight mass spectrometer (St. Joseph, MI) operated in positive electron ionization mode at 70 eV, in high resolution (25,000) (full-width-at-half-maximum) with a mass range *m*/*z* 30-650, 5 spectra/s, and at a source temperature of 200°C. Samples were introduced with an Agilent 7693

autosampler with a 7890B GC equipped with a multimode inlet operated in cold splitless mode onto a GC column (RXi-5ms, 30 m × 0.25 mm i.d., 0.25 μm film thickness, Restek, Bellefonte, PA). The GC temperature program began at an initial temperature of 35°C (4 min), increased at 9 °C/min to 280 °C, and was held for 30 min. The transfer line was held at 280 °C and the injection port at 250 °C.[9] Analyses were performed in full scan.

Mass spectra of unknown compounds were subjected to library database searching (using the National Institute of Standards and Technology [NIST] database. When there was not a sufficient library match to offer an identification, high resolution data, when available, was used to provide empirical formulas for molecular ions and fragments. Additionally, mass spectra were also subject to manual interpretation, where fragmentation and isotope patterns were used to provide tentative structural identifications. To confirm tentative identifications, standards were purchased or synthesized and used to compare mass spectra and retention times.

North Carolina Water Reaction Conditions. For comprehensive identification of iodo-DBPs in North Carolina water from ICM, reactions were conducted by a collaborator. To summarize their research methods, approximately 121 L of source water from the Cape Fear River (total organic carbon: 2.1 ppm, [Br^-/I^-]: <0.5 μM) was reacted headspace free, buffered with

phosphate buffer to a pH of 7.5. Iopamidol was spiked at 5μM and HOCl (disinfectant) was spiked at a concentration of 100 μM. (Blank: North Carolina water with disinfectant, no iopamidol.) This light sensitive reaction was carried out in large barrels (with Teflon liners) for 72 hours. An example chromatogram obtained from North Carolina water is illustrated in Figure 3.9.

South Carolina Water Reaction Conditions. For comprehensive identification of iodo-DBPs in South Carolina water from ICM, 6.5 L of source water from the Broad River was collected in 20L Teflon carboys and filtered once through Merck Millipore Glass Fibre prefilters (5 μm) and then through Merck Millipore Durapore Membrane filters (0.45 μm). (Prior to extraction and analysis, water was stored at 4°C in the dark) The water was buffered with a 10 mM borate buffer to a pH of 8.5. Iopamidol was added at a concentration of 1 μM and HOCl was added at a concentration of 12 ppm. (Blank: South Carolina water with iopamidol, no disinfectant). The reaction was carried out for 24 hours. An example chromatogram obtained from South Carolina water is illustrated in Figure 3.10.

Germany Water Reaction Conditions. For comprehensive identification of iodo-DBPs in Germany water from ICM, 10 L of source water from the Rhine River was filtered through Merck Millipore Glass Fibre 5 μm prefilters and then through Merck Millipore Durapore Membrane 0.45 μm filters. The water was

then buffered with a 10 mM borate buffer to a pH of 8.5. Iopamidol was spiked at a concentration of 1 μM and HOCl was spiked at a concentration of 12 mg/L or 3 mg/L. (Blank: Rhine River water with disinfectant, no iopamidol). The reaction was carried out for 1 hour, 4 hours, 8 hours, or 24 hours. An example chromatogram obtained from Germany water is illustrated in Figure 3.11.

I-THMs Quantification Experiments. Analytical methods were created to measure iodo-trihalomethanes (I-THMs), a group of priority unregulated DBPs. Stock solutions of DBP standards were made by dissolving DBP standards in anhydrous acetonitrile or methanol. A calibration curve for I-THMs was made with the following concentrations: 0.005, 0.010, 0.025, 0.050, 0.100, 0.500, 1.00, 2.50, 5.00, and 10.0, 20.0, 30.0, 40.0, and 50.0 μg/L.

Analyte detection was performed using electron ionization (EI) on an Agilent 5975 inert XL Mass Selective Detector mass spectrometer equipped with an Agilent model 6890 GC and operated at an accelerating voltage of 8 kV and a source temperature of 200°C (Santa Clara, CA), carried out in selected ion monitoring (SIM) mode. Table 3.5 shows selected ions (*m*/*z* values) and retention times selected to monitor each compound. Injections of 1 μL of the extracts were introduced via a split/splitless injector (in pulsed splitless mode) onto a GC column (RTX-200, 30 m X 0.25 mm i.d., 0.25 μm film thickness, Restek, Bellefonte, PA). The GC temperature program consisted of an initial temperature of 35°C (4

min), increased to 9°C/min to 280°C and held for 30 minutes. The transfer line was held at 280°C and the inlet temperature at 250°C with an electron energy at 70 eV. Quantification ions had a dwell time of 100 ms, and qualifier ions had a dwell time ranging from 50 to 75 ms. Quantification ions were selected based on relative abundance, generally selecting the most abundant ions.

South Carolina Water I-THMs Quantification Experiments. To measure volatile I-THMs in South Carolina water, a single liquid–liquid extraction (LLE) was performed. The water was first filtered once through Merck Millipore Glass Fibre prefilters (5 μm) and then through Merck Millipore Durapore Membrane filters (0.45 μm) and buffered to a pH of 8.5 using 10 mM borate buffer. Iopamidol at a concentration of 1 μM and chlorine at a concentration of 12 ppm were added to the water. The water was reacted headspace free in the dark for 24 hours. After 24 hours elapsed and prior to the extraction, the amount of residual, unreacted chlorine was measured using the DPD (N, N-diethyl-p-phenylenediamine) method.[65]. Samples were then quenched with excess ascorbic acid (chlorine to ascorbic acid molar ratio of 1:1.3) and adjusted to pH 3.5–4 with 1 M sulfuric acid. Aliquots of 100 mL were spiked with 30 g of sodium sulfate and 2 mL of MTBE in 125 mL amber bottles. Samples were shaken for 30 min on a mechanical shaker, followed by a 10 min resting period to allow the organic phase and aqueous phase to separate. The organic phase was then removed and

deposited into a conical test tube. Anhydrous sodium sulfate was added to the extract to remove any excess water, and 250 μL was transferred with a syringe into a gas chromatography (GC) vial. Final extracts were spiked with internal standard 1,2-dibromopropane.[1]

Germany Water I-THM Quantification Experiments. To measure volatile I-THMs in Germany water, a single liquid–liquid extraction was performed. The water was first filtered once through Merck Millipore Glass Fibre prefilters (5 μm) and then through Merck Millipore Durapore Membrane filters (0.45 μm) and buffered to a pH of 8.5 using 10 mM borate buffer. Iopamidol at a concentration of 1 μM and chlorine at a concentration of either 3 ppm or 12 ppm were spiked into the water. The water was reacted headspace free in the dark for different amounts of time (TABLE 3.6). After the designated period of time for the given experiment had elapsed and prior to the extraction, the amount of residual, unreacted chlorine was measured using the DPD (N, N-diethyl-p-phenylenediamine) method.[65]. Samples were then quenched with excess ascorbic acid (chlorine to ascorbic acid molar ratio of 1:1.3) and adjusted to pH <1 with 1 M sulfuric acid. Aliquots of 100 mL were spiked with 30 g of sodium sulfate and 2 mL of MTBE in 125 mL amber bottles. Samples were shaken for 30 min on a mechanical shaker, followed by a 10 min resting period to allow the organic phase and aqueous phase to separate. The organic phase was then removed and

deposited into a conical test tube. Anhydrous sodium sulfate was added to the extract to remove any excess water, and 250 μL was transferred with a syringe into a gas chromatography (GC) vial. Final extracts were spiked with internal standard 1,2-dibromopropane.[1]

IAAs Quantification Experiments. Analytical methods were created to measure iodo-acetic acids (IAAs), a group of unregulated priority DBPs. Stock solutions of DBP standards were made by dissolving DBP standards in methanol. One calibration curve for IAAs was made with the following concentrations: 0.005, 0.010, 0.025, 0.050, 0.100, 0.500, 1.00, 2.50, 5.00, and 10.0, 20.0, 30.0, 40.0, and 50.0 μg/L.

Derivatized samples were analyzed by GC-tandem mass spectrometry (MS/MS) for iodoacetic acids using a Thermo TSQ-MS triple quadrupole mass spectrometer coupled to a TRACE GC 2000 gas chromatograph (Thermo Scientific, Waltham, MA). Sample volumes of 2.0 μL were injected at an inlet temperature of 200°C with a splitless time of 0.50 min and split flow of 10 mL/min. GC separations were performed using an RTX-5 column (30 m × 0.25 mm ID × 0.25 μm film thickness; Restek Corporation, Bellefonte, PA**)**, with the following oven temperature program: 35°C for 4 minutes, followed by a 9°C/min ramp to 280°C, and held for 20 min. The transfer line temperature was controlled at 200°C. An EI source was used at a temperature of 180°C,

emission current of 50 μA, and electron energy of 70 eV. Multiple reaction monitoring (MRM) was used to quantify iodocetic acid (IAA), chloroiodoacetic acid (CIAA), bromoiodoacetic acid (BIAA), and diiodoacetic acid (DIAA). Two MS–MS transitions, one quantitative and one qualitative, were used for each of the IAAs, along with 1,2-dibromopropane internal standard (Figure 3.12, Table 3.7). Table 3.7 also shows the optimized collision energies for each of the iodo-acids quantified.

South Carolina Water IAAs Quantification Experiments. To measure volatile IAAs in South Carolina water, a multiple liquid–liquid extraction (LLE) was performed. The water was first filtered once through Merck Millipore Glass Fibre prefilters (5 μm) and then through Merck Millipore Durapore Membrane filters (0.45 μm) and buffered to a pH of 8.5 using 10 mM borate buffer. Iopamidol at a concentration of 1 μM and chlorine at a concentration of 12 ppm were spiked into the water. The water was reacted headspace free in the dark for 24 hours. After 24 hours elapsed and prior to the extraction, the amount of residual, unreacted chlorine was measured using the DPD (N, N-diethyl-p-phenylenediamine) method.[65]. Samples were then quenched with excess ascorbic acid (chlorine to ascorbic acid molar ratio of 1:1.3) and adjusted to pH <1 with concentrated sulfuric acid. Aliquots of 100 mL were spiked with 30 g of sodium sulfate and 5 mL of MTBE in 125 mL amber bottles. Samples were shaken for

15 min on a mechanical shaker, followed by a 10 min resting period to allow the organic phase and aqueous phase to separate. The organic phase was then removed and deposited into a conical test tube. Samples were extracted again for a total of three LLEs and a total of 15 mL of MTBE. The collected extract was passed through a sodium sulfate column to remove excess water, and concentrated under nitrogen to a final volume of 200 μL. Final extracts were derivatized using 100 μL of freshly generated diazomethane, as previously described.

3.3 Results and Discussion

North Carolina Water. Figure 3.9 shows the GC-MS chromatogram of the North Carolina water that was spiked with iopamidol and chlorine disinfectant. In this chromatogram, the *m*/*z* 126.9 peak was extracted (I^+ fragment) for de-convolution purposes. Of the peaks extracted, several were identified as iodo-DBPs. Two new compounds, chloroiodoacetonitrile and iodoacetonitrile, that have never before been reported as DBPs, were found. These compounds were identified using their characteristic mass spectra (Figures 3.13- 3.16). Because they had not been reported as DBPs prior to this study, the samples were re-analyzed on a high resolution time-of-flight GC-MS (HR-TOF-GC-MS) (Figures 3.17 and 3.18). High resolution allows for accurate mass data to be obtained, which is extremely useful in identifying unknowns. The high resolution mass

spectrum for chloroiodoacetonitrile gave an observed *m*/*z* of 200.8836 as compared to the theoretical *m*/*z* of 200.8842. The *m*/*z* 200.8836 and *m*/*z* 202.8836 peaks result from the two natural isotopes of chlorine (^{35}Cl and ^{37}Cl). The peak at *m*/*z* 126.9039 was identified as iodine, which has an exact mass of 126.9044. The peak at *m*/*z* 73.97910 was identified as C_2HNCl^+, which has an exact mass of 73.97975. We are currently waiting for a standard of chloroiodoacetonitrile to be synthesized for confirmation. The iodoacetonitrile peak was confirmed using a standard and the mass spectra was also compared to a library spectra for additional support. The observed *m*/*z* from the high resolution spectra was *m*/*z* 166.9226 as compared to the theoretical mass of *m*/*z* 166.9232.

Also present in the North Carolina water chromatogram was a peak identified as iodoacetic acid. Usually, halo-acids are not seen in the underivatized samples. They usually exist at too low of concentrations to be detected without derivatization. However, in this sample, the concentration was high enough where it could be identified in its underivatized form. This is significant because iodoacetic acid is the most toxic DBP known to date (Figure 3.19). The toxicity of iodoacetonitrile had been studied prior to this experiment. While it is not as toxic as iodoacetic acid, it is more toxic than the known animal carcinogen, 3-chloro-4-(dichloromethyl)-5-hydroxy-5*H*-furan-2-one , more commonly known as Mutagen X (MX). [27] Because there is not currently a

standard for chloroiodoacetonitrile, the toxicity is unknown. However, the toxicity is predicted to be similar to that of iodoacetonitrile.

Using a new highly sensitive GC-MS/MS method, several new tri-halo acids were detected in the North Carolina waters. This method has low ppt detection levels, achieved through multiple reaction monitoring of predicted fragmentations for the compounds (Figure 3.12). Currently, we are waiting for standards of the tri-halo acids to be synthesized for confirmation.

South Carolina Water. Figure 3.10 shows the GC-MS chromatogram of the South Carolina water that was spiked with iopamidol and chlorine disinfectant. In this chromatogram, the *m*/*z* 126.9 peak was once again extracted. The two new compounds, chloroiodoacetonitrile and iodoacetonitrile, found in the iopamidol reactions with North Carolina waters were also identified in these reactions. Iodoacetic acid was also present again in its underivatized form.

Several I-THMs were quantified in the South Carolina water that was reacted with iopamidol and chlorine for 24 hours. Dichloroiodomethane was found at a concentration of 2.9 ± 0.22 μg/L, bromochloroiodomethane was found at a concentration of 0.38 ± 0.0091 μg/L, dibromoiodomethane was found at a concentration of 0.38 ± 0.0011 μg/L, and chlorodiiodomethane was found at a concentration of 0.56 ± 0.00042 μg/L (Figure 3.20). None of these compounds were found at detectable concentrations in the blank (South Carolina water

reacted with chlorine disinfectant, no iopamidol), suggesting they are the byproduct of the reaction between iopamidol and chlorine.

Several iodo-acids were formed in the South Carolina water after the 24 hour reaction of iopamidol with chlorine disinfectant. Iodoacetic acid was present at 131.2 ± 50.3 ng/L, chloroiodoacetic acid was present at 144.8± 42.5 ng/L, and diiodoacetic acid was present at 4.2 ± 1.7 ng/L. None of these acids were present at detectable concentrations in the blank (South Carolina water reacted with chlorine, no iopamidol), suggesting that they are formed in the reaction between iopamidol and chlorine (Figure 3.21).

Germany Water. Figure 3.11 shows an example GC-MS chromatogram of Rhine River water from Germany spiked with iopamidol and chlorine disinfectant with the *m*/*z* 126.9 peak extracted. No iodo-THMs or iodo-acids were identified in this analysis using GC-MS will full-scan. This is likely because the levels detected were too low to be observed in a full scan analysis. Several I-THMs were observed and quantified by the instrument analyzing in SIM mode, which is a more sensitive method of analysis.

Dichloroiodomethane, bromochloroiodomethane, and dibromoiodomethane were found to form when Rhine River water was reacted with iopamidol and chlorine in various timed reactions (Figures 3.22-3.24). Levels for chlorodiiodomethane (CDIM), iodoacetonitrile (IAN),

bromodiiodomethane (BDIM), and triiodomethane (TIM), were non-detect for all time points. Specifically, dichloroiodomethane (DCIM) was quantified in the water that was reacted with iopamidol and 3 mg/L of chlorine for just ten minutes at a concentration of 0.470 ± 0.0035 μg/L. It was quantified in every subsequent reaction of iopamidol with 3 mg/L of chlorine, with a maximum concentration of 0.83 ± 0.0063 μg/L for the 24 hour reaction (Figure 3.22). DCIM was also measured at a level of 0.61 ± 0.064 μg/L in the water reacted with 12 mg/L of chlorine and iopamidol for 24 hours. The concentration of DCIM formed was less with 12 mg/L of chlorine, and it also took significantly longer for DCIM to form at this concentration of disinfectant. This is potentially because the excess chlorine inhibits the formation of DBPs. Most DBPs formed from chlorine disinfectant are formed through oxidation and substitution reactions. These reactions proceed much more rapidly at high pH than low pH.[66] In the water spiked with 12 mg/L of chlorine, the higher concentration of chlorine is achieved by adding more hypochlorous acid. The increased concentration of acid lowers the pH, which can slow the reactions that form I-THMs. Thus, higher concentrations of I-THMs are observed when the waters were spiked with 3 mg/L of chlorine over 12 mg/L of chlorine.

Bromochloroiodomethane (BCIM) was also quantified in several samples of Rhine River water reacted with iopamidol and chlorine. It was first observed

in the sample reacted for 30 minutes, at a concentration of 0.44 ± 0.0012 μg/L and observed in every subsequent sample spiked with 3 mg/L of chlorine (Figure 3.23). The greatest concentration was observed in the sample reacted for 24 hours. BCIM was also formed after 8 hours in the sample spiked with 12 mg/L of chlorine at a concentration of 0.45 ± 0.0091 μg/L and in the sample spiked with 12 mg/L of chlorine and reacted for 24 hours at a concentration of 0.45 ± 0.015 μg/L. The trend of increased formation with 3 mg/L of chlorine as compared to 12 mg/L of chlorine was observed.

Lastly, dibromoiodomethane (DBIM) was quantified in two samples; it was quantified in the sample reacted for 20 hours at a concentration of 0.35 ± 0.00064 μg/L, and in the sample reacted for 24 hours at a concentration of 0.35 ± 0.00071 μg/L (Figure 3.24). This limited data suggests that somewhere between 8 and 20 hours dibromoiodomethane is formed from the reaction of iopamidol and chlorine in Rhine River water. The maximum concentration is reached no later than 20 hours, as seen by the similar concentrations observed at both 20 and 24 hours. Again, the trend of increased formation with a lower concentration of disinfectant was observed.

Because none of the I-THMs were present in the water reacted for 0 minutes, it can be concluded that all of the I-THMs found were formed from the

reaction of iopamidol with disinfectant and natural organic matter in the water from Germany.

In the Germany water experiments to quantify I-THMs, water was found present in the MTBE extracts. Because not all of the water was removed in the extraction, these experiments should be repeated for more reliable quantifications.

3.4 Conclusions

It is important to identify and quantify iodo-DBPs because they are the most toxic class of DBPs and none of them are currently regulated. There are low MW iodo-DBPs formed from the reaction of iopamidol with natural organic matter and disinfectant. Some of these low MW iodo-DBPS, such as iodoacetic acid, are already known to be toxic. This supports the hypothesis that lower MW iodo-DBPS are contributing to the increase in toxicity when iopamidol is present in the water. It can be concluded that X-ray contrast media are able to serve as iodinated precursors in the formation of toxic iodo-DBPs.

This work comprehensively identified fifteen different iodo-DBPs in three different source waters from North Carolina, South Carolina, and Germany that were reacted with iopamidol and chlorine disinfectant (Table 3.8). Several of these iodo-DBPs were found for the first time. A new GC-MS/MS method was used to identify some of these iodo-DBPs because it has low ppt detection levels

and is therefore very sensitive. Several I-THMs were quantified at low μg/L (ppb) levels after having formed in South Carolina water reacted with iopamidol and chlorine for 24 hours. Additionally, several iodoacetic acids were also quantified in this water at low ppt levels. In the water from Germany, it was found that In general, higher levels of I-THMs were formed in waters spiked with 3 mg/L of chlorine vs. waters spiked with 12 mg/L of chlorine. This was attributed to the increased pH when 12 mg/L of chlorine was spiked, slowing down the formation of I-THMs. Higher concentrations were generally found in waters that had been reacted the longest. All concentrations were found to be low μg/L levels.

FIGURES

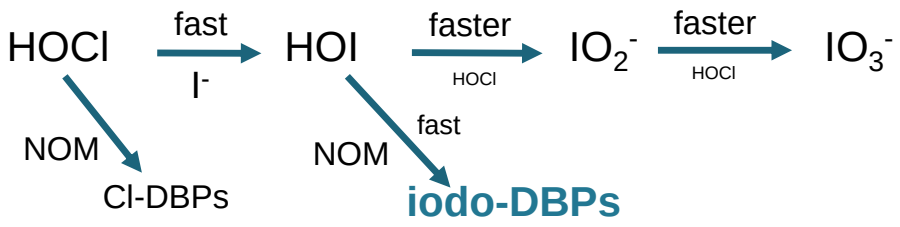

Figure 3.1: Formation of DBPs when chlorine is used as the disinfectant.[58]

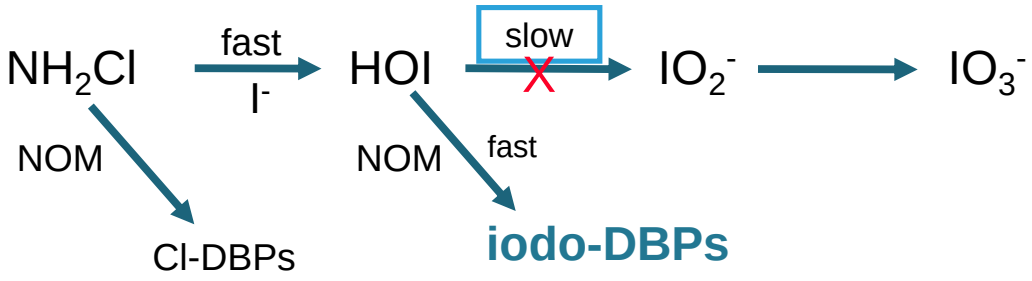

Figure 3.2: Formation of DBPs when monochloramine is used as the disinfectant.[58]

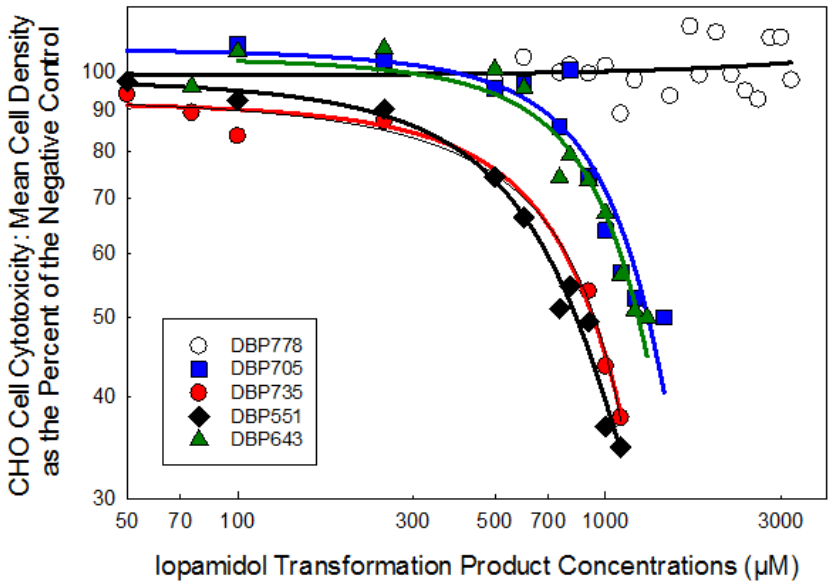

Figure 3.3: Cytotoxicity of high MW iopamidol transformation product iodo-DBPs. Note: None were genotoxic.[61]

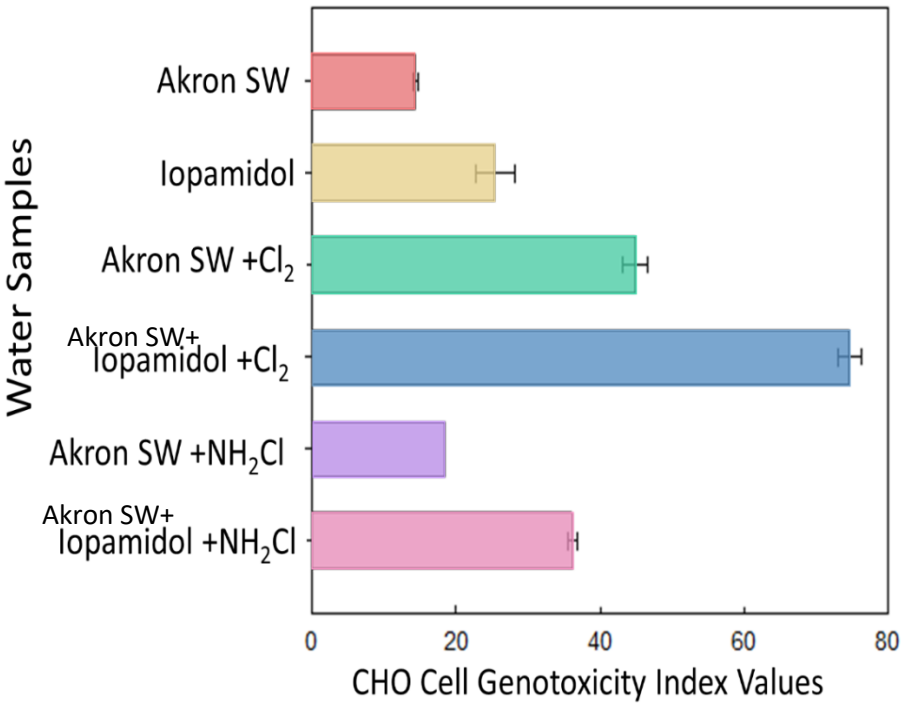

Figure3.4: Increased genotoxicity when iopamidol is present.

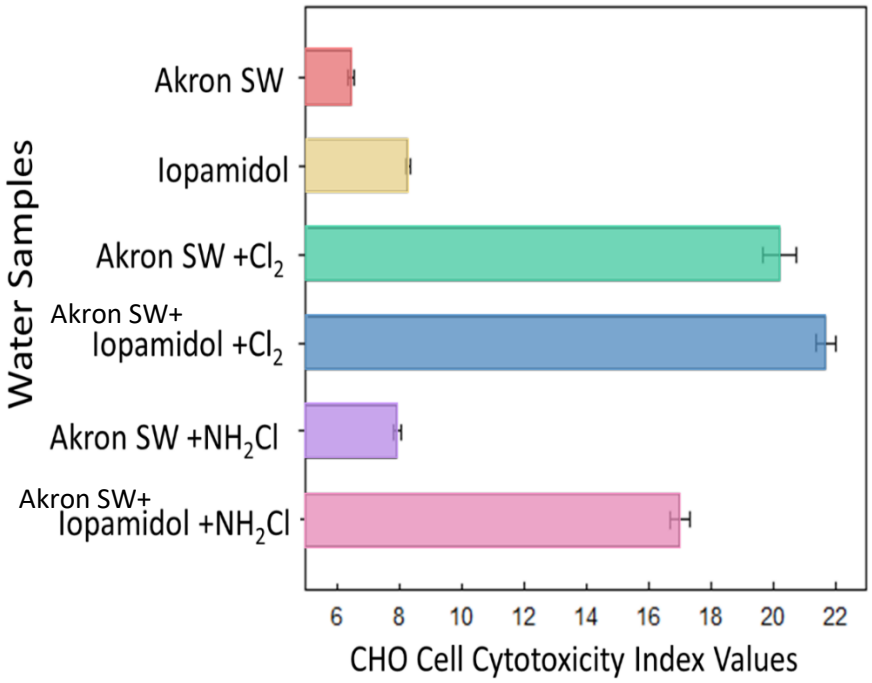

Figure 3.5: Increased cytotoxicity when iopamidol is present.

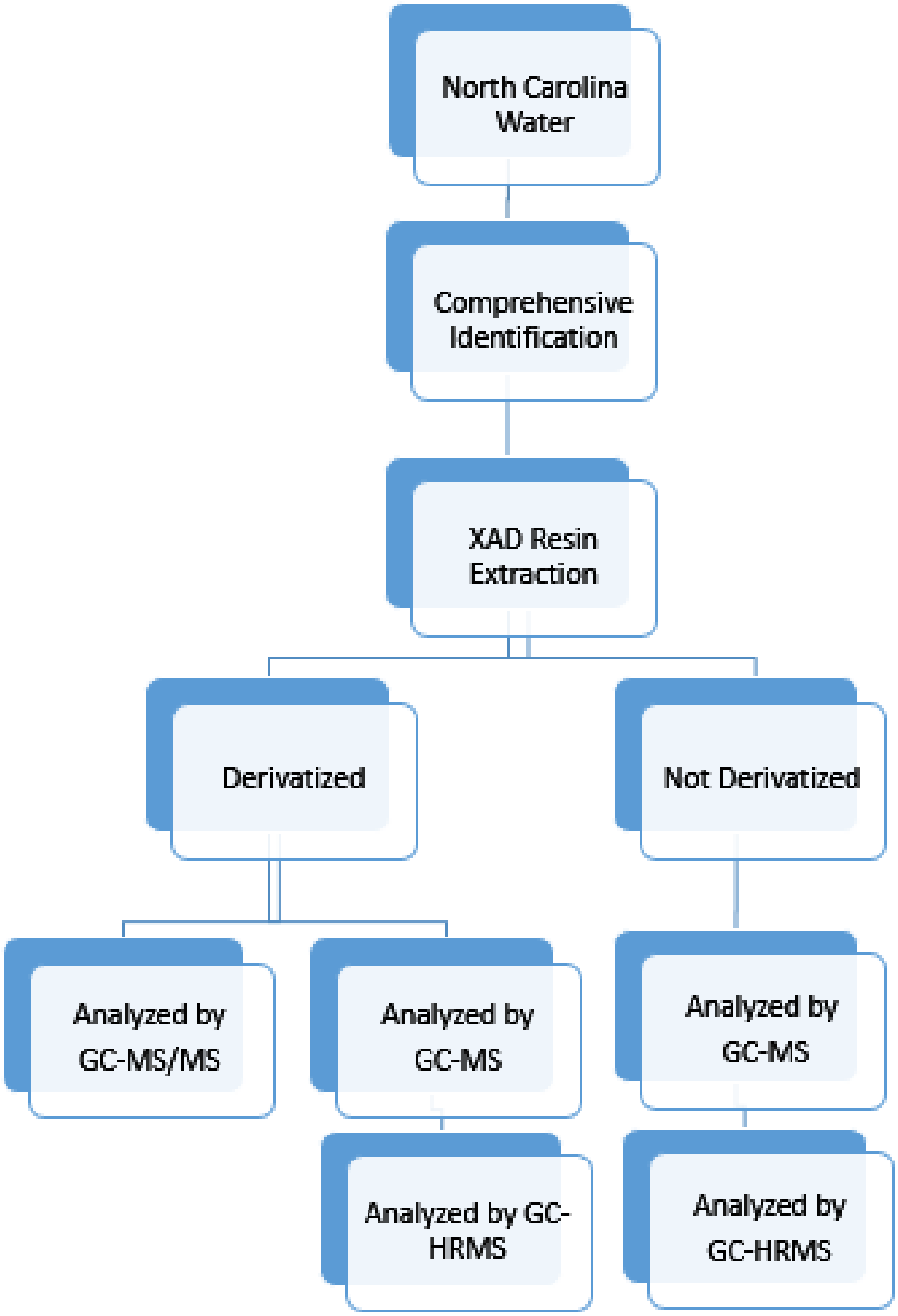

Figure 3.6: Summary of experiments performed on North Carolina river water from the Cape Fear River.

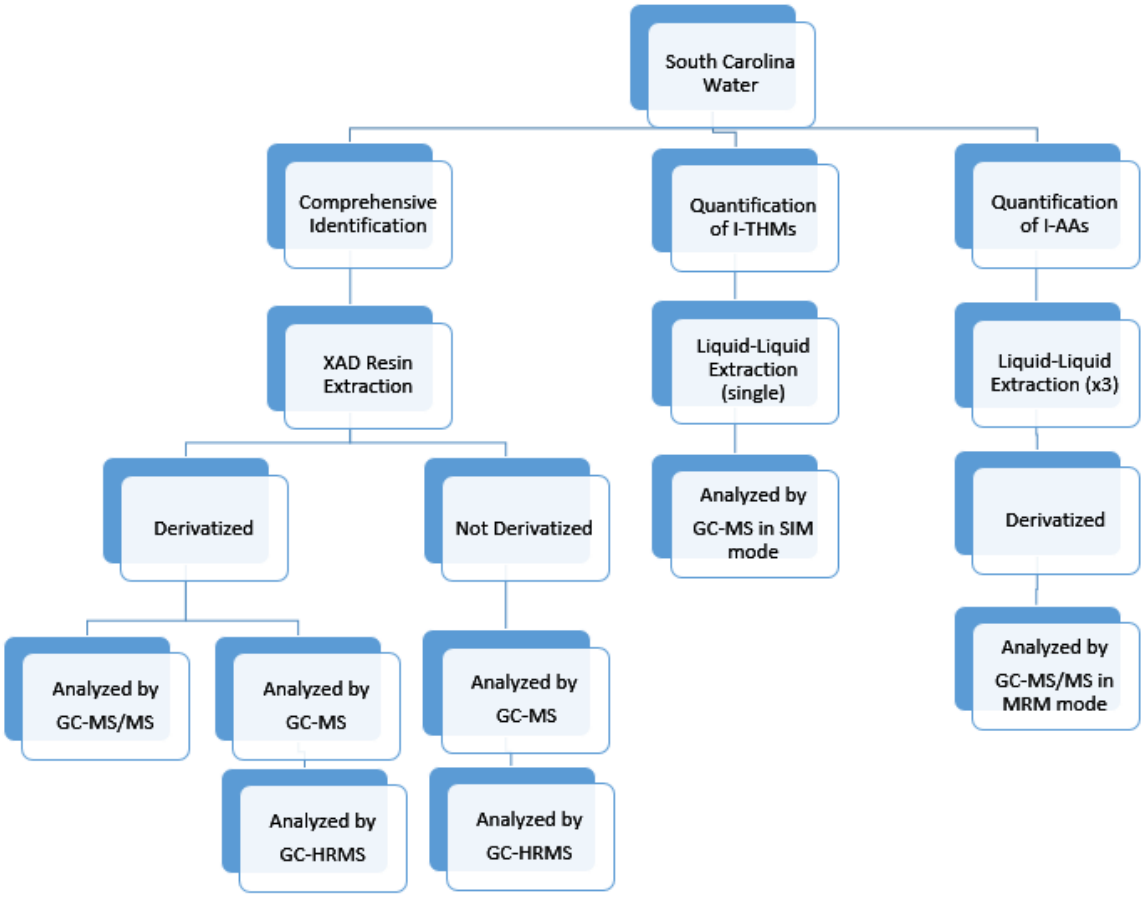

Figure 3.7: Summary of experiments performed on South Carolina river water from the Broad River.

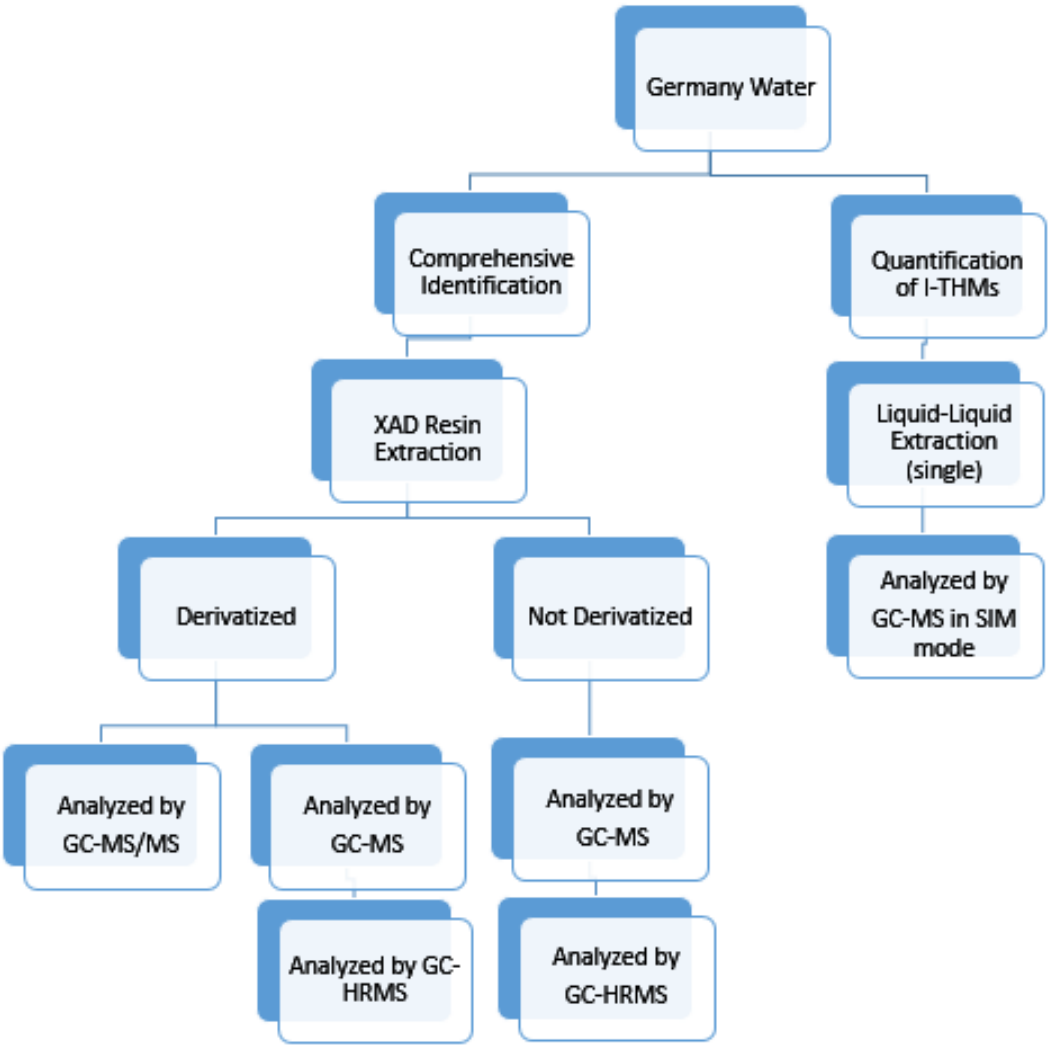

Figure 3.8: Summary of experiments performed on Germany river water from the Rhine River.

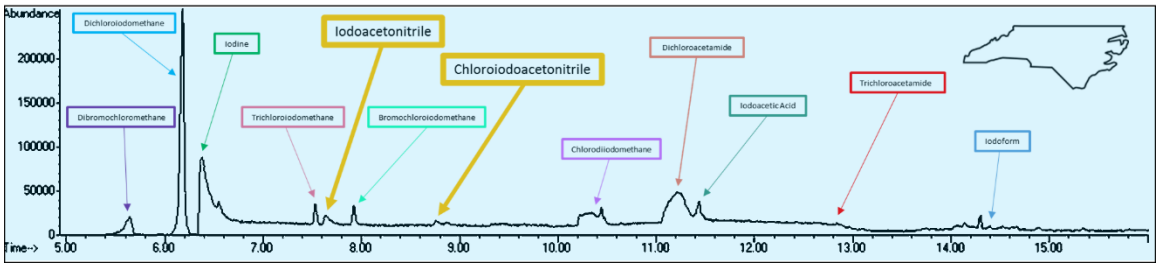

Figure 3.9: GC chromatogram of North Carolina river water with iodo-DBPs identified. m/z 126.9 extracted.

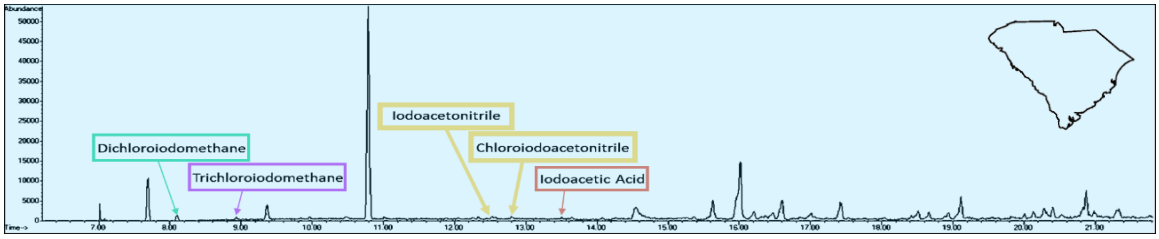

Figure 3.10: GC chromatogram of South Carolina river water with iodo-DBPs identified. m/z 126.9 extracted.

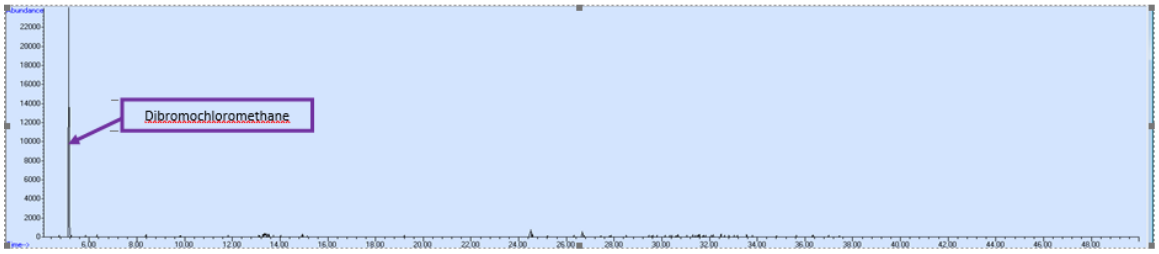

Figure 3.11: GC chromatogram of Germany river water with iodo-DBPs identified. m/z 126.9 extracted.

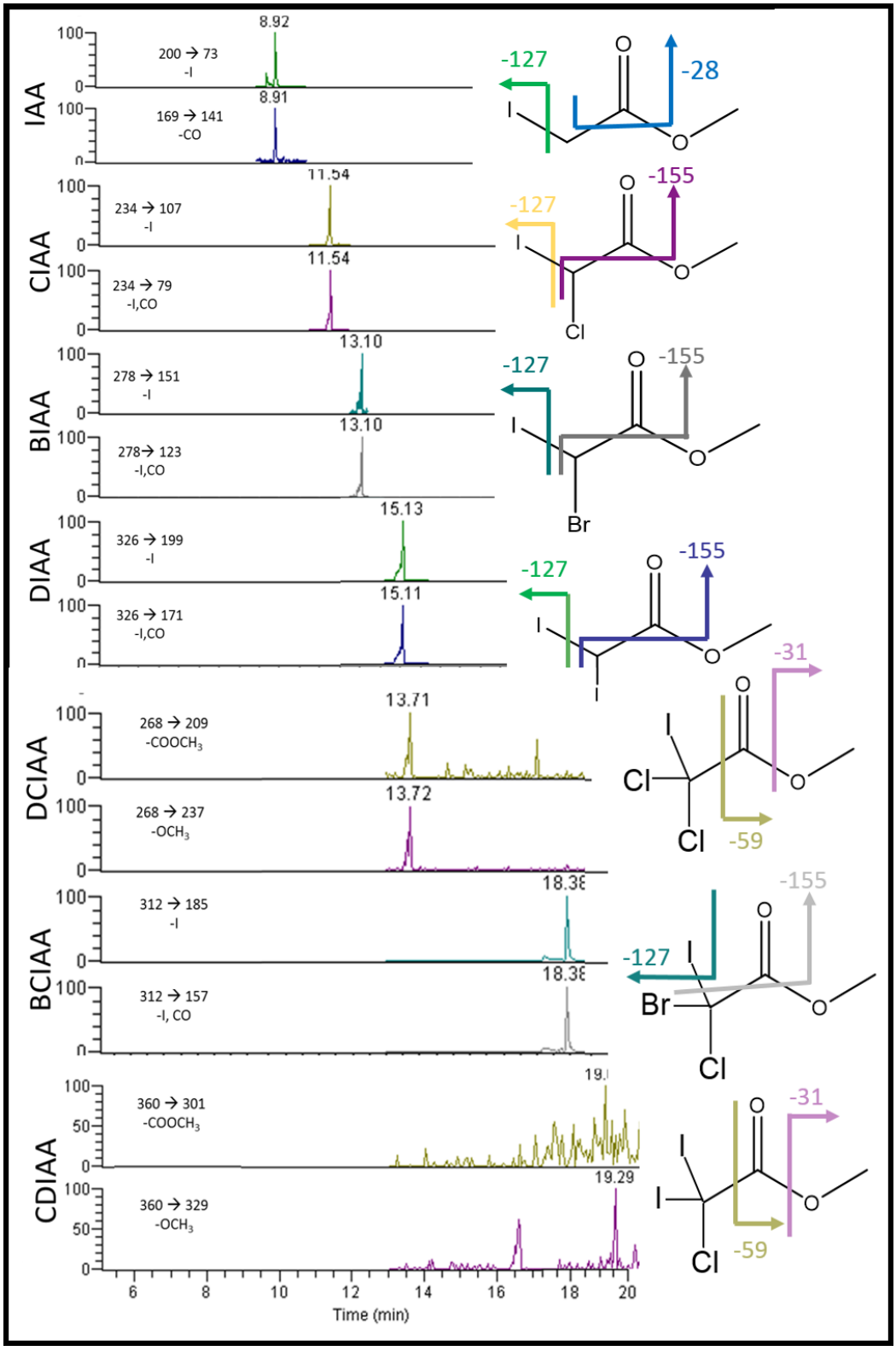

Figure 3.12: MRM transitions for IAAs quantified and tentatively identified new trihalo-IAAs.

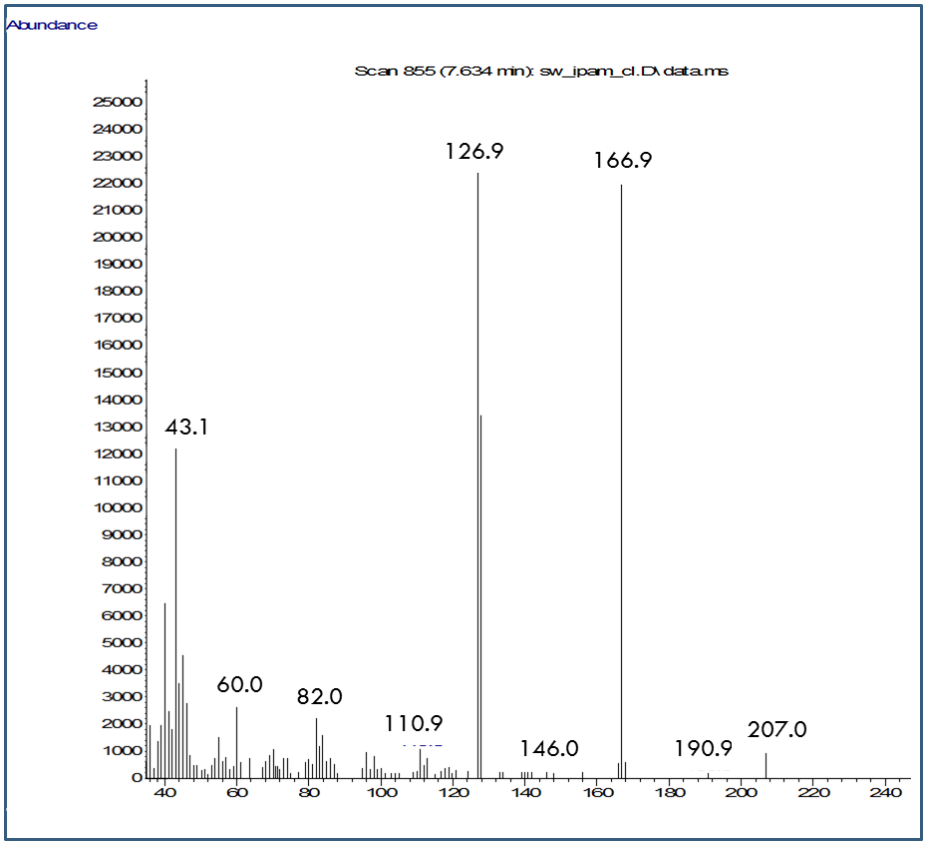

Figure 3.13: Low resolution mass spectrum of iodoacetonitrile.

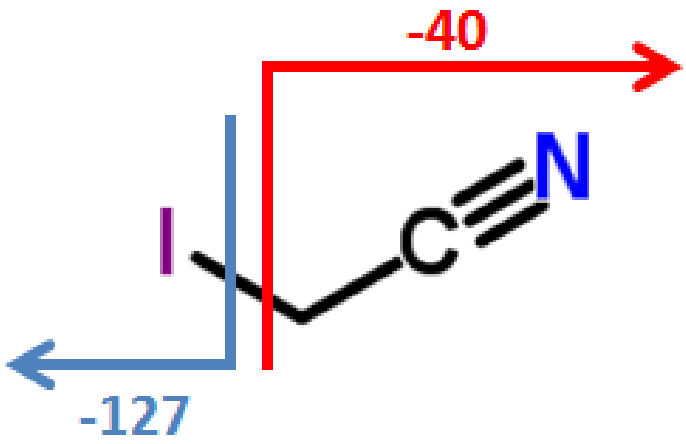

Figure 3.14: Structure and mass fragments of iodoacetonitrile.

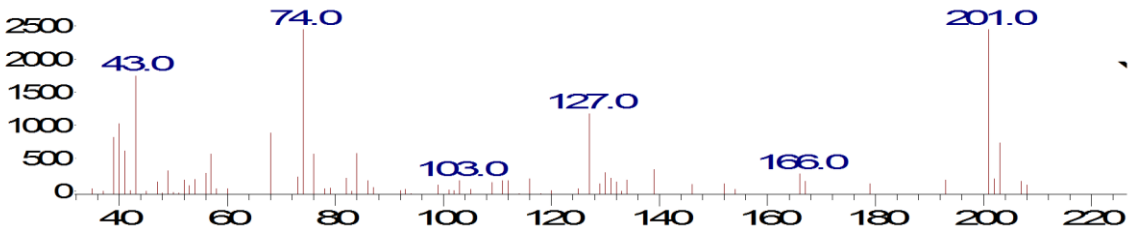

Figure 3.15: Low resolution mass spectrum of chloroiodoacetonitrile.

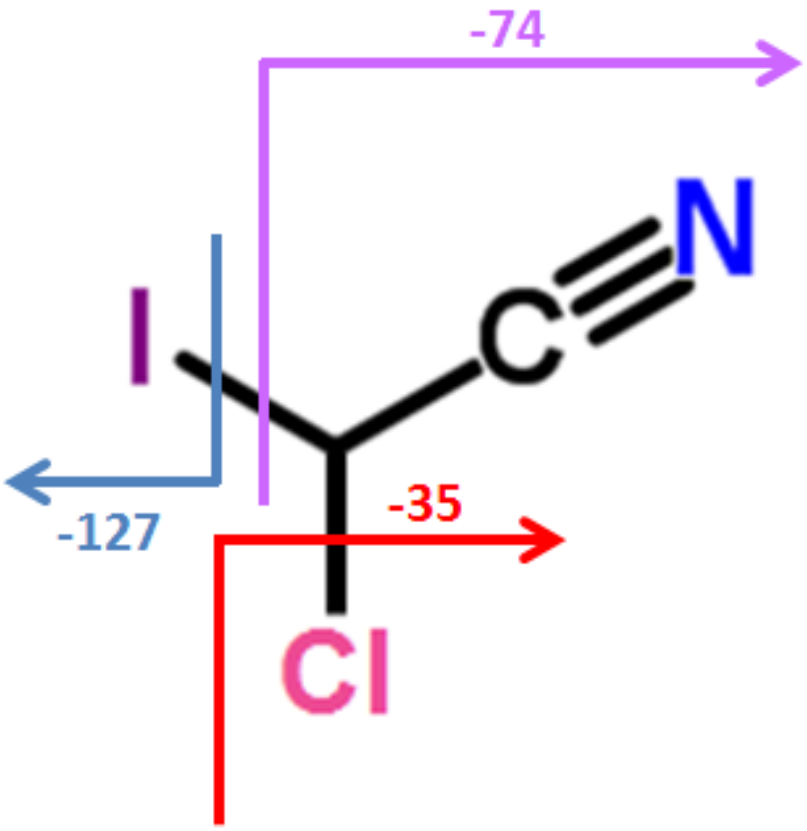

Figure 3.16: Structure and mass fragments of chloroiodoacetonitrile.

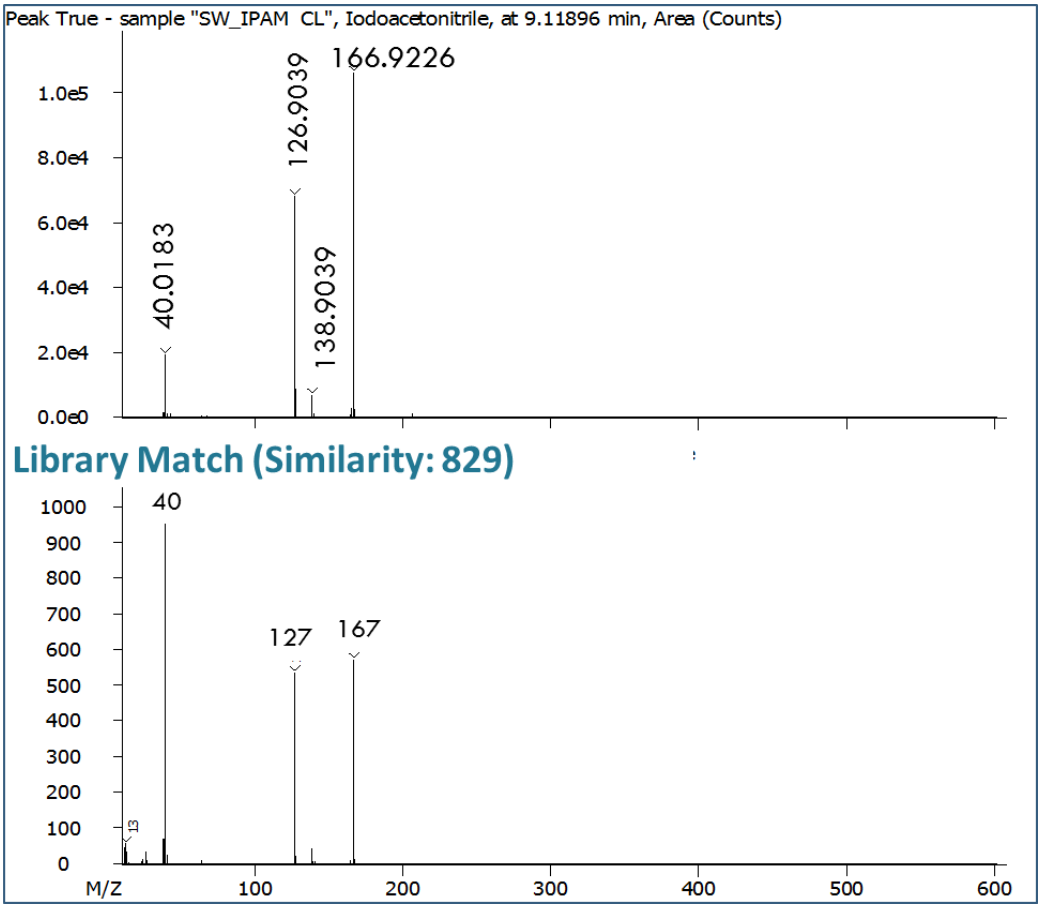

Figure 3.17: High resolution mass spectrum of iodoacetonitrile with library match.

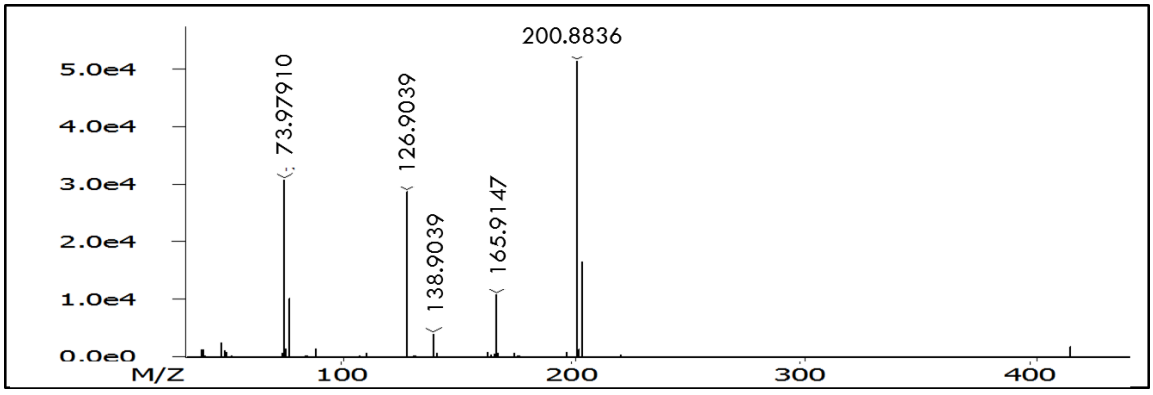

Figure 3.18: High resolution mass spectrum of chloroiodoacetonitrile.

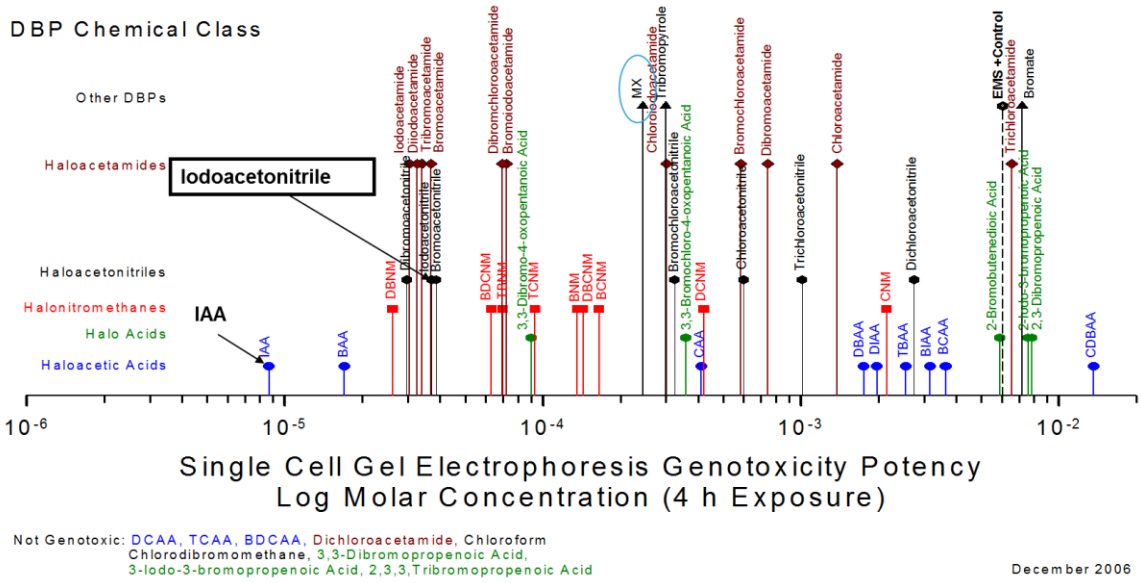

Figure 3.19: Genotoxicity of DBPs by class. Iodoacetonitrile highlighted.[67]

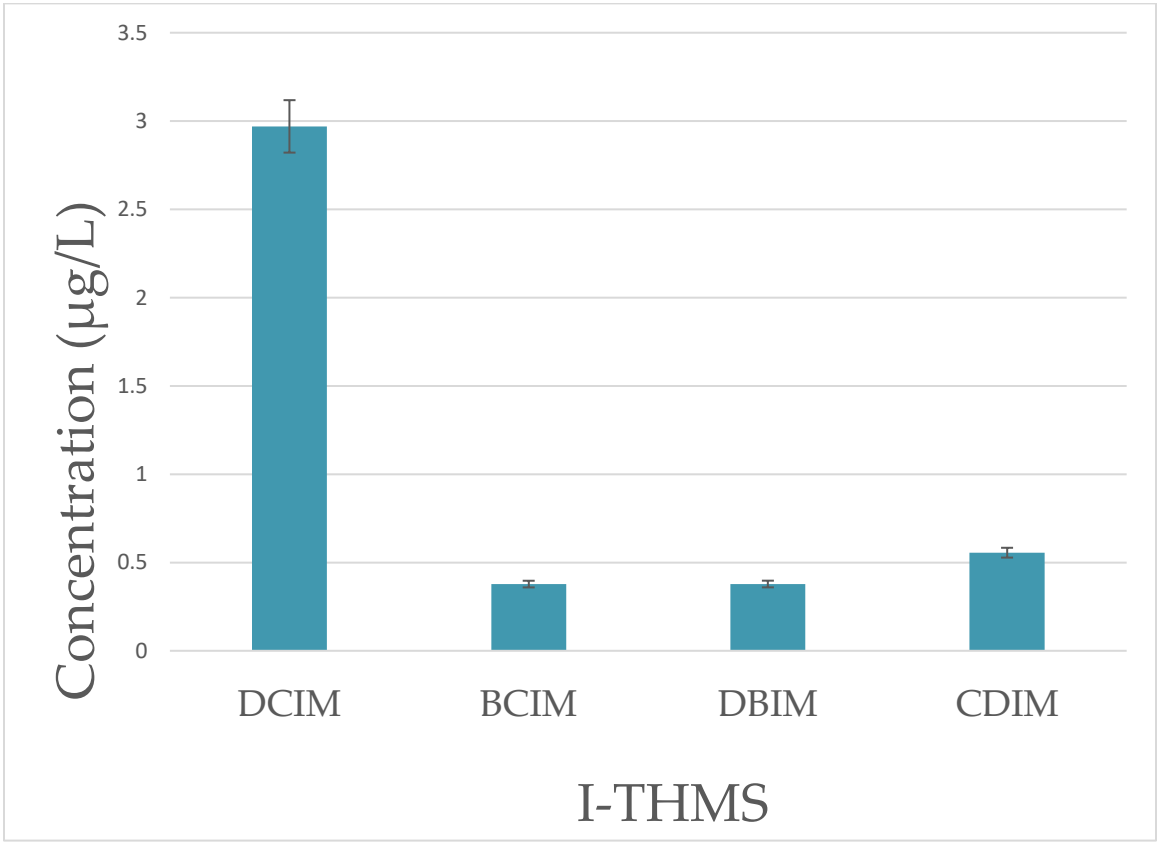

Figure 3.20: Concentrations of iodo-THMs in South Carolina river water reacted with iopamidol and chlorine.

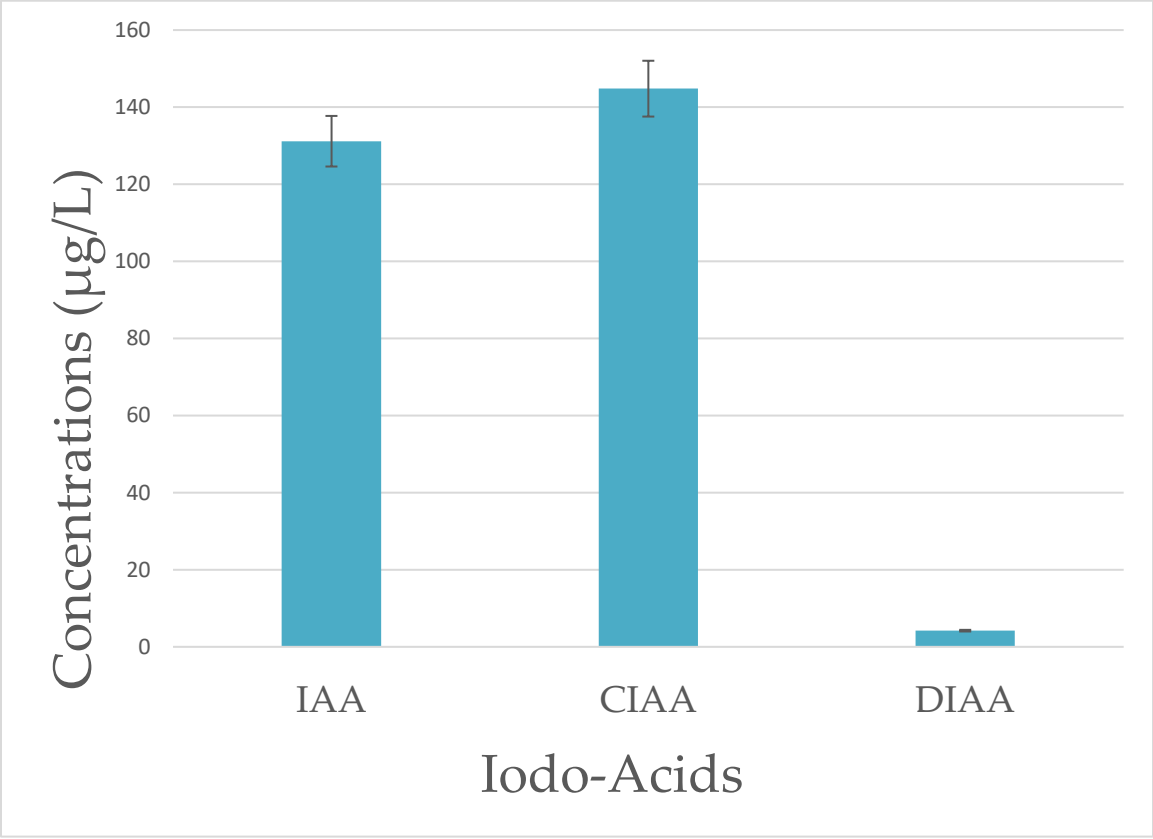

Figure 3.21: Concentrations of iodo-acids in South Carolina river water reacted with iopamidol and chlorine.

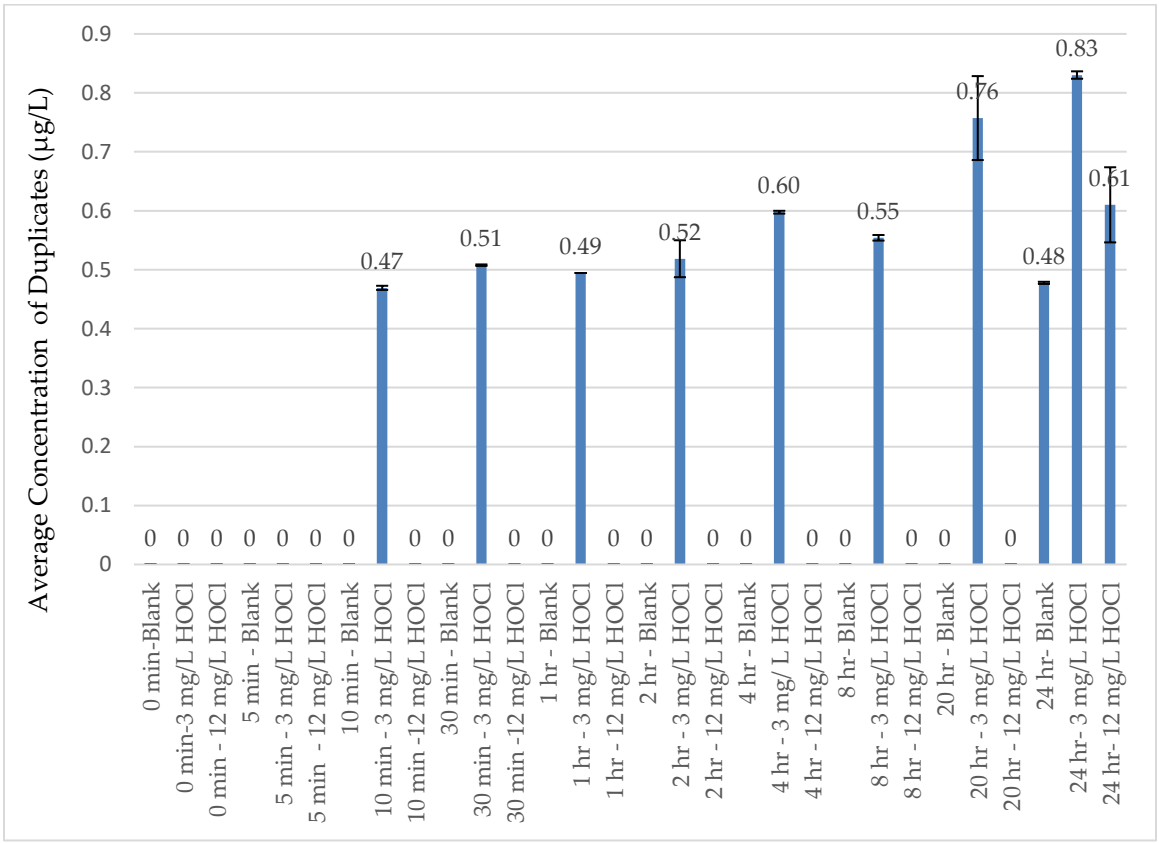

Figure 3.22: Concentrations of DCIM for various timed reactions of iopamidol and chlorine in Germany river water.

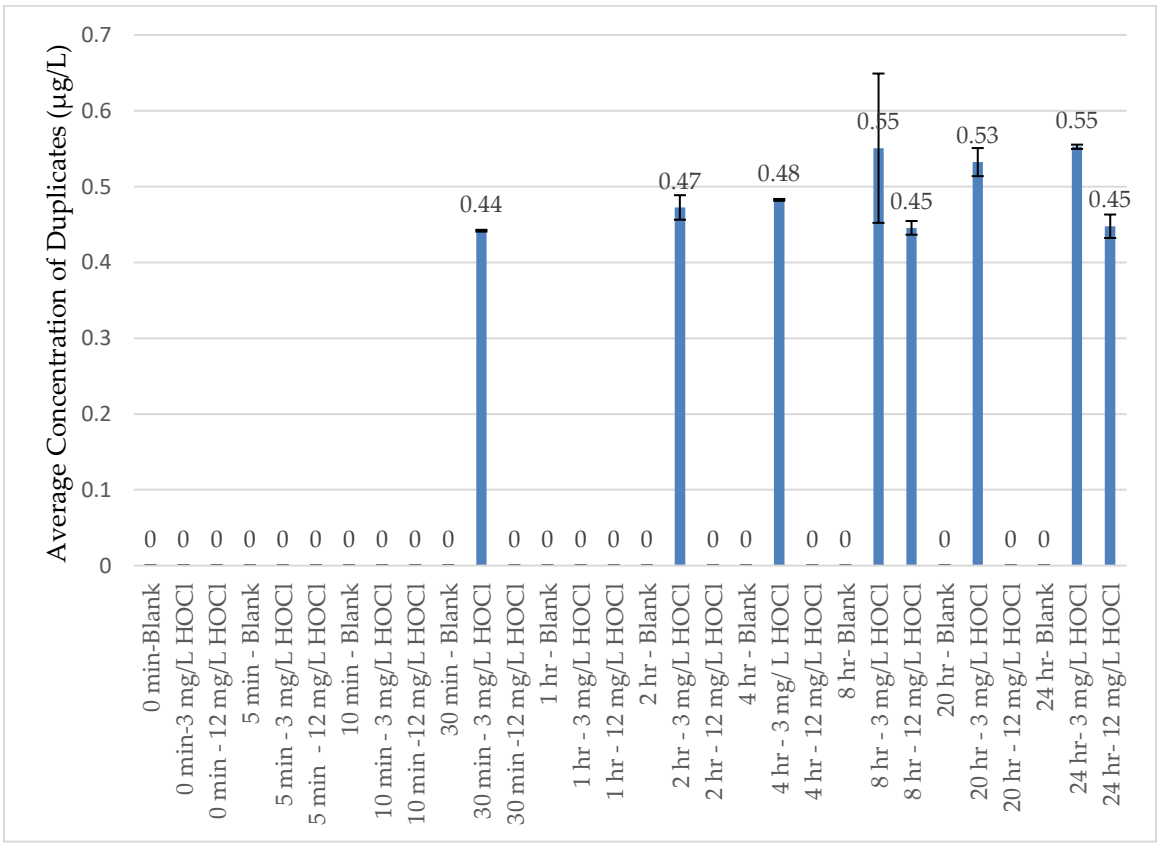

Figure 3.23: Concentrations of BCIM for various times reactions of iopamidol and chlorine in Germany river water.

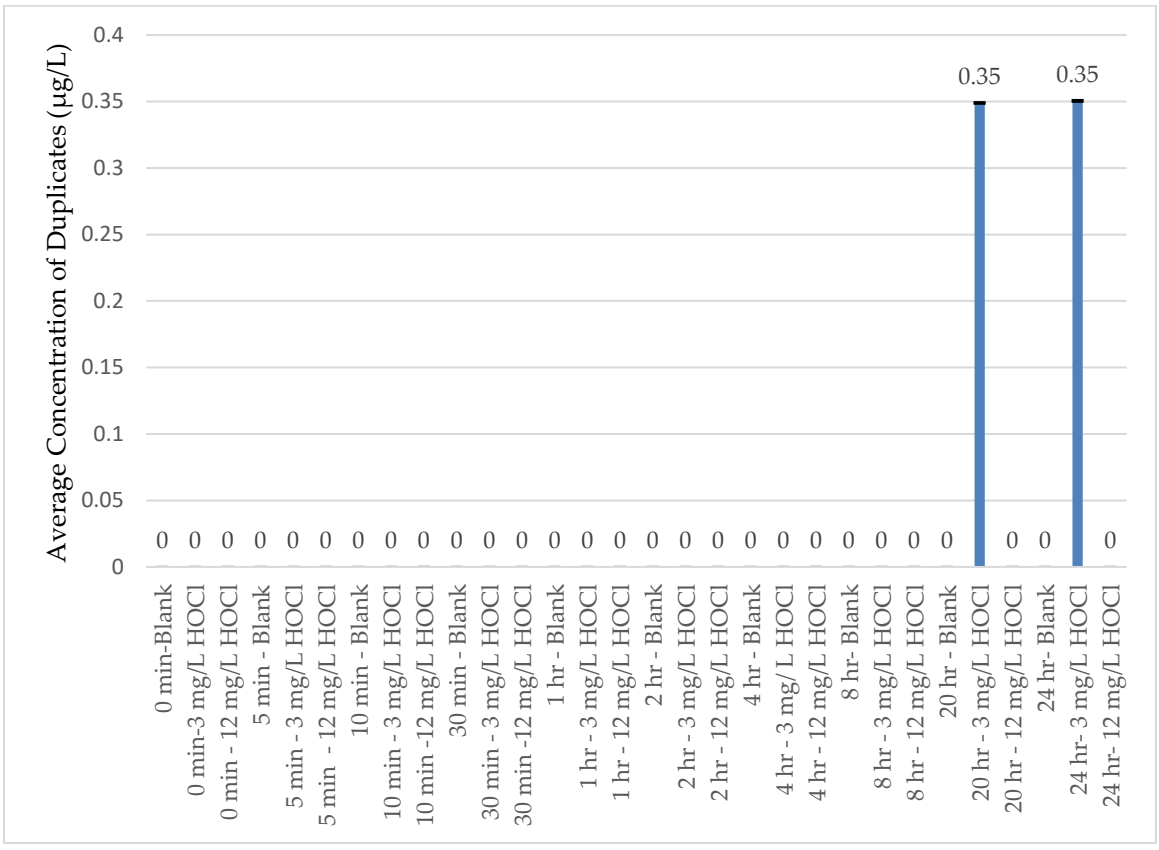

Figure 3.24: Concentrations of DBIM for various timed reactions of iopamidol and chlorine in Germany river water.

TABLES

Table 3.1: Four cities in the 23-city occurrence study with low levels of naturally occurring iodide, but high levels of iodo-DBPs.[1]

	Iodide Naturally Present (µg/L)	Σ Iodo-Acids (µg/L)	Σ Iodo-THMs (µg/L)
City 2	1.0	0.37	4.9
City 4	ND	0.10	1.2
City 11	1.5	0.21	2.3
City 15	ND	0.17	2.4

Table 3.1: Concentrations (ppt) of ICM in four cities of 23-city occurrence study.[59]

	Iopamidol	Iomeprol	Iopromide	Iohexol	Diatrizoate
City 2	510	ND	24	120	93
City 4	110	ND	6	49	ND
City 11	100	ND	ND	85	ND
City 15	2700	ND	25	ND	ND

Table 2.3: Mammalian cell toxicity of high molecular weight iodo-DBPs vs. low molecular weight iodo-DBPs.

High Molecular Weight DBPs	LC_{50} (μM)	Low Molecular Weight DBPs	LC_{50} (μM)
DBP705	1441	Iodoacetic Acid	2.95
DBP735	934	Diiodoacetic Acid	332
DBP51	832	Bromoiodoacetic Acid	897
DBP643	1296	Iodoform	66
DBP778	Not Toxic	Dibromoiodomethane	4130
		Dichloroiodomethane	4130
		Bromochloroiodomethane	2400
		Chlorodiiodomethane	2410

Table 3.3: Reaction conditions for North Carolina, South Carolina, and Germany river waters.

	North Carolina Reaction Conditions	South Carolina Reaction Conditions	Germany Reaction Conditions
Volume	121 L	6.5 L	10 L
Buffer	KH_2PO_4/KH_3HPO_4 10 μM	Borate 10 μM	Borate 10 μM
pH	7.5	8.5	8.5
[Iopamidol]	5 μM	1 μM	1 μM
$[Cl_2]$	100 μM	12 ppm	12 ppm
Reaction Time	72 Hours	24 Hours	1 Hour 4 Hours 8 Hours 24 Hours
XAD	45 mL XAD2 45 mL XAD 8 420 mL of Ethyl Acetate Eluted	30 mL XAD2 30 mL XAD 8 200 mL of Ethyl Acetate Eluted	30 mL XAD2 30 mL XAD 8 200 mL of Ethyl Acetate Eluted

Table 3.4: Selected ions and retention times on RTx-200 column for SIM acquisition mode of some target iodo-DBPs.

DBP	RTx-200 SIM (mins)	Major Ions (m/z)
DCIM	8.308	83/85/126.9
DBP (Internal Standard)	10.601	121/123
BCIM	10.613	126.9/128.9
DBIM	12.704	172.8/299.7
CDIM	13.256	174.9/302
IAN	14.035	167/126.9
BDIM	15.574	218.8/220.8
TIM	18.853	266.8/393.7

Table 3.5: Summary of timed I-THM quantification experiments of Germany water.

Experiment/ Reaction Time	0 min	5 min	10 min	30 min	1 hr	2 hr	4 hr	8 hr	20 hr	24 hr
Blank (no iopamidol, 12 mg/L chlorine)	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL
1 µM Iopamidol, 3 mg/L chlorine	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL
1 µM Iopamidol, 12 mg/L chlorine	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL

Table 3.6: Segment times, transitions, and optimized collision energies for some iodo-acids.

Segment	Compound	Time	Transitions (*m*/*z*)	Collision Energy
1	1,2-Dibromopropane (Internal Standard)	8 mins	121 → 41	9 eV
			123 → 41	9 eV
2	Iodoacetic acid methyl ester	8-10.5 mins	200→ 73	8 eV
			169 → 141	8 eV
3	Chloroiodoacetic acid methyl ester	10.5-12.5 mins	234→107	4 eV
			234→79	12 eV
4	Bromoiodoacetic acid methyl ester	12.5-14 mins	278 → 151	6 eV
			278 → 123	12 eV
5	Diiodoacetic acid methyl ester	14-20 mins	326 → 199	6 eV
			326 → 171	12 eV

Table3.7: Summary of iodo-DBPs found in reacted waters.

Iodo-THMs	Iodo-Nitriles	Iodo-Acids
Dibromoiodomethane	Chloroiodoacetonitrile	Bromochloroiodoacetic Acid
Dichloroiodomethane	Iodoacetonitrile	Bromoiodoacetic Acid
Bromochloroiodomethane		Chlorodiiodoacetic Acid
Trichloroiodomethane		Chloroiodoacetic Acid
Chlorodiiodomethane		Dichloroiodoacetic Acid
Iodoform		Diiodoacetic Acid
		Iodoacetic Acid

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